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The Microrna Profile of Granular Cell Tumours

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Granular cell tumours were first described by Abrikossoff in 1926 as lesions derived from smooth muscle, and referred to as granular cell myoblastoma. However, the exact histogenesis of the tumour is debatable, with some evidence suggesting a neural cell of origin. The purpose of this study was to examine the microRNA (miRNA) expression profile of granular cell tumours when compared to normal skin and to determine if the histogenesis of granular cell tumours can be clarified with their miRNA profile. MicroRNAs are small non-coding RNAs that down regulate gene expression in cellular apoptosis, differentiation, development, and appear to be expressed in a cell lineage specific manner. To address our question, we began by comparing the miRNA expression profiles of granular cell tumours (n = 8) to normal skin (n = 2) using archival formalin fixed, paraffin embedded (FFPE) tissue and the Agilent miRNA microarray platform. Then, using Genespring software, we found both up-regulated and down-regulated miRNAs in the granular cell tumours relative to normal skin. Firstly, our results showed that unsupervised hierarchical clustering was able to separate the granular cell tumours from normal skin. The three most significantly down-regulated miRNAs were: miR-203, showing a 315-fold change ($p \leq .05$), miR-200c, showing a 227-fold change ($p \leq .05$), and miR-200b, showing a 116-fold change ($p \leq .05$) in granular cell tumours when compared to their expression in normal skin. The three most significantly up-regulated miRNAs were: miR-370, showing a seven-fold change ($p \leq .05$), miR-490-5p, showing a six-fold change ($p \leq .05$), and miR-23a, showing a 5.6-fold change ($p \leq .05$) in granular cell tumours when compared to their expression in normal skin. The reduction of the miR-200 family has been previously shown to correlate with epithelial-mesenchymal transition (EMT) and thus with granular cell tumours, this finding is in line with its mesenchymal differentiation. We plan to compare the granular cell tumour profile with that of smooth muscle and neural tissue to better elucidate its lineage. In summary, this is the first description of the miRNA profile of granular cell tumours, and this work may lead to a better understanding of the histogenesis of this enigmatic tumour.

Expression of the DNA Mismatch Repair Proteins Hmlh1 and Hmsh2 in Pancreatic and Small Intestinal Neuroendocrine Tumours

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Background: While the role of the MEN1 gene in the pathogenesis of some gastrointestinal neuroendocrine tumours (NETs) is well known, different genetic and epigenetic pathways must be responsible for the pathogenesis of tumours with intact MEN1. Two recent small studies have identified a high degree of microsatellite instability (MSI-H) or reduced expression of the DNA mismatch repair protein hMLH1 in a proportion (10–33%) of pancreatic NETs, suggesting that a significant subset of these tumours arise due to defects in the DNA mismatch repair apparatus. MSI-H status was identified very rarely in intestinal NETs in two other small studies. Loss of expression of hMLH1/hMSH2 by immunohistochemistry (IHC) is known to correlate strongly with MSI-H status in colorectal adenocarcinoma. Immunohistochemical expression of hMLH1 and hMSH2 has not been well studied in pancreatic or intestinal NETs.

Objective: To study IHC expression of hMLH1 and hMSH2 in pancreatic and intestinal NETs.

Design: Seventy-one patients (32 male, 39 female, mean age 59.1 years) were identified who had a resection for a primary pancreatic NET (n = 34) or small intestinal NET (n = 37) over an 18 year period at the QEII HSC in Halifax, NS. Archived tissue samples from all patients were placed in tissue microarrays. Immunostains for hMLH1 and hMSH2 were applied and assessed.

Results: There was preserved expression of hMLH1 and hMSH2 in the nuclei of all 34 pancreatic NETs. 35 of 37 small intestinal NETs had preserved hMLH1 expression. One tumour was negative and one was indeterminate for hMLH1 expression due to absent hMLH1 staining in both the tumour and internal control cells. All 37 small intestinal NETs had preserved hMSH2 expression.

Conclusions: We do not identify any loss of hMLH1 or

hMSH2 expression in pancreatic NETs detectable by IHC. Defects in DNA mismatch repair seem rare in pancreatic and intestinal NETs.

An In-Depth Study of Extraprostatic Extension and Margin Status in Radical Prostatectomies

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Objectives: To determine the prognostic significance of (1) extraprostatic extension (EPE) +/-positive margins and vice versa (2) focal vs non-focal EPE, and (3) different EPE subtypes.

Methods: Slides from 148 radical prostatectomies (93-01) were reviewed and pathological features (Gleason score, PCa volume, SVI, LVI, LN metastasis, margin status, EPE including focality, subtype) and clinical data (biochemical failure, metastasis, survival) were recorded.

Results: 1. Prognostic Significance of EPE+/-Positive Margins and Vice Versa

2. EPE Focality: Non-focal EPE is present in 89% of metastasis and 89% of deaths due to PCa.

	+EPE only (n = 47)	+Margin Only (n = 23)	+EPE & Margin (n = 38)	Control (n = 40)	p-Value
%Biochemical Failure (3-160 mo)	42	28	58	13	<.001
%Metastasis	19	0	24	0	<.001
%Survival (1–6 yrs)	91	100	85	100	.027

3. EPE Subtypes: Tumour in fat is present in 94% of metastasis and 89% of deaths due to PCa. Tumour in neurovascular bundle is present in 72% of metastasis & 78% of deaths due to PCa.

Conclusions: Positive EPE regardless of margin status is an important predictor of metastasis and death due to PCa, whereas, a positive margin in the absence of EPE is not. Thus, more importance should be given to EPE than margins when it comes to determining prognosis and treatment. Also non-focal EPE is far more common in cases of metastasis and death than focal EPE. Additionally, of the EPE subtypes, tumour in fat is the most consistent predictor of metastasis and death.

Automated Image Analysis of Microvascular Density in Clear Cell Renal Cell Carcinoma and Its Prognostic Utility

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Objectives: The clinical significance of tumour microvascular density (MVD) in clear cell renal cell carcinoma (ccRCC) is debated. Previous attempts to assess MVD in ccRCC have been limited by manual quantification of MVD within only a small area of tumour. To overcome this, we used standardized image analysis, and normalized the data by assessing the cellular density of the tumour; we then assessed the MVD of ccRCC and compared it with clinical outcome data.

Methods: Clinico-pathologic data was collected for 50 cases of ccRCC. Each tumour and adjacent normal kidney was immunostained for CD34, Ki-67, and VEGF and whole slide scanned using an Aperio CS Scanner (Vista, CA) at 20× magnification. Computer image analysis was then used to assess MVD and nuclear density. We generated sets of raw and normalized vascular data and tested these for correlations with clinical prognostic parameters and survival data.

Data and Results: Tumours with increased MVD showed a statistically significant association with higher tumour stage compared to tumours with lower vascularity (p = .017). Increased MVD showed a statistically significant correlation with poor prognosis and a decrease in progression free survival versus those tumours with low MVD (p = .016). There was a positive correlation between MVD and tumour size (RS = 0.456; p =.001).

Conclusions: The latest developments in digitized histology can be used for objective standardized assessment of prognostic markers. Our standardized assessment revealed that increased MVD in ccRCC was associated with higher tumour stage and size and a decreased disease free survival. These results may prove useful in the development of new prognostic tests and anti-angiogenic strategies.

Clinical Significance of Mir-21 in Renal Cell Carcinoma

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Background: Renal cell carcinoma (RCC) is the most common neoplasm of the adult kidney. Increasing evidence suggests that miRNAs are dysregulated in RCC and are important players in RCC pathogenesis. MiR-21 is a known oncogene with tumour promoting effect in many types of cancer. Its role in RCC is yet to be elucidated.

Design: We analyzed miR-21 in RCC. 105 cases of renal cell carcinoma and controls were retrieved from St. Michael's and Sunnybrook hospitals including 10 papillary, 10 chromophobe, 10 oncocytoma, 10 metastatic and 65 primary clear cell RCC. Total RNA was extracted from formalin fixed paraffin embedded tissues using a modified protocol, followed by reverse transcription. The expression of miR-21 was measured with a Real-Time PCR using TaqMan miR-21-specific probes. Results were normalized using a small RNA RNU 44 as an internal control.

Results: The expression of miR-21 is increased four-fold in renal cell carcinoma. There is a difference in the expression levels between subtypes with the highest level of expression in clear cell and papillary subtypes. Also, expression levels show significant differences among different stages and between primary and metastatic tumours, and correlates with survival.

Conclusion: There is increased expression of miR-21 in renal cell carcinoma. Its level of expression can be used as a diagnostic marker of the tumour subtype and also as a prognostic marker.

Development of an Evidence-Based Approach to Proficiency Testing Assurance for Breast Cancer Biomarker Immunohistochemistry: Definition of Reference Values

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Background: Laboratory participation in external quality assurance (EQA) programs proficiency testing for immunohistochemical assessment of breast cancer biomarkers is necessary to achieve optimal results. At present, various reference values are used by EQA programs.

Design: The purpose of this study was to explore different reference values for ER, PR and HER2 IHC, in a tissue microarray-based EQA proficiency testing program (CIQC): consensus values (51% or 80 % overall agreement between all participants), consensus value or majority result as determined in four reference laboratories, and agreement with the labs using FDA-approved method for ER/PR or FISH for HER2). Participating laboratories stained a single tissue microarray slide containing 44 breast carcinomas for each biomarker and submitted it for interpretation. All results were subjected to statistical analysis using five reference values for each sample, as defined above; concordance rates, sensitivity, specificity, positive and negative predictive values and kappa values were calculated for each lab and biomarker.

Result: Some laboratories showed consistently suboptimal performance of their ER and/ or PR stainings, based on their respective evidence-based parameters, no matter what reference value was used for calculations. Comparison of the reference values used showed near perfect agreement. For HER2, when ASCO (2007) guidelines were used for comparison (score 2 excluded), all but one lab showed excellent concordance. When score 2 was included as a positive score (as a criterion for FISH testing), there was

poor agreement between the majority of the participating labs. Nevertheless, the laboratories with suboptimal performance were detected by most of reference methods used.

Conclusion: Reference values in immunohistochemistry EQA should be defined individually for each biomarker. FDA-approved kits may be used in addition to consensus results from participating laboratories and reference laboratory results. While there are advantages to the use of multiple reference check-points to objectively assess laboratory performance in EQA proficiency testing, all of the above methods can identify poor performance.

Bilateral Renal Sinus Myelolipomas: Case Report and Review of the Literature

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Introduction: Myelolipomas are benign neoplasms consisting of hematopoietic cellular elements and adipose tissue that classically arise in the adrenal glands. They are uncommon and are found in only 0.4–1% of the population at autopsy. Extra-adrenal myelolipomas (EMs) are extremely rare with fewer than 50 cases reported.

Case Report: A 62-year-old female presented with a history of vague abdominal pain. MRI of the abdomen revealed three masses in the left renal sinus (largest 10.2 cm) and two similar masses in the right renal sinus. Percutaneous biopsies of the largest lesion on the left side revealed predominantly fat cells with aggregates of mature hematopoietic cells and their precursors. The differential diagnosis included EM and extramedullary hematopoiesis (EH). Active surveillance was initiated, however the largest left sided mass grew and the patient became increasingly symptomatic. Following a negative hematologic work-up, the left kidney was removed. Histological examination of the final surgical specimen revealed EM arising from the left renal sinus.

Discussion: We describe the first reported case of bilateral EMs of the renal sinus detected by MRI. The literature on EMs is reviewed from the perspectives of sites of origin, differential diagnosis, pathogenesis and natural history.

A Study of TdT Expression in Merkel Cell Carcinoma and Pulmonary Neuroendocrine Carcinomas

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Background: Merkel cell carcinoma (MCC) is an uncommon tumour with indistinct clinical features. Despite their rarity, the differential diagnosis includes pulmonary neuroendocrine carcinomas (PNC) which simulate MCCs histologically. The aim of this study is to characterize the level and pattern of expression of terminal deoxynucleotidyl transferase (TdT) and an updated panel of immunohistochemical (IHC) markers that may assist in the differential diagnosis between MCC and PNC.

Methods: MCC and PNC cases accessioned between 1995–2009 were retrieved from the archives of Sunnybrook Health Sciences Centre. Two pathologists reviewed the cases and studied the IHC profile using a panel of antibodies against: TdT, thyroid transcription factor 1 (TTF-1), c-KIT (CD117), HER-1 epidermal growth factor receptor (EGFR), cytokeratin 7 (CK7), cytokeratin 20 (CK20), chromogranin (Chrom) and synaptophysin (Syn). Immunostaining was recorded semiquantitatively.

Results: We identified 33 MCC and 13 PNC cases. The following percentages of positive cases are indicated in the table below. MCC staining with TdT was diffuse and strong. Conclusions: Our study confirms the strong expression of TdT and CK20 in MCC. We demonstrate that TdT may be a useful reagent for the diagnosis of MCC and for assisting in distinguishing between MCC and PNC. These findings suggest that a set of three IHC stains, TdT, TTF-1, and CK20 are useful in providing a distinction between MCC and PNC.

Antibody	TdT	TTF-1	CK7	CK20	CD117	HER-1	Chrom	Syn
MCC	76	9	6	88	88	15	79	100
PNC	7	54	39	8	77	54	77	85

Correlation of Immunohistochemical Expression Levels of Alpha-3 and Alpha-1 Subunits of Sodium Pump with Prostate Cancer Prognosis: A Tissue Microarray Study

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Objective: Expression of the α subunit of the sodium pump (Na^+/K^+ATPaseα) is altered in many cancer types. We hypothesized there is an association between the expression of α-1 (SUα1) and α-3 (SUα3) subunits and prostate cancer (PCA) prognosis.
Methods: We selected men diagnosed with a pT3N0-2M0 PCA and treated by radical prostatectomy. Percentage of cancer cells expressing SUα1 and SUα3 was evaluated by immunohistochemistry on a tissue microarray. Frequency tables with Pearson's correlations were built to evaluate the association between the expression levels of SUα1, SUα3, SUα3:SUα1 ratio and known PCA prognostic factors. Wald test from Cox regression model was used to measure the association between SUα1, SUα3, SUα3:SUα1 ratio and time to biochemical recurrence and death of any causes.
Results: Mean follow-up time of the 74 men was 13 years. Thirty-two biochemical recurrences and 8 deaths were observed. SU 1 expression level was inversely associated with Gleason score ($p = .0019$). SUα3 and SUα3:SUα1 ratio were not associated with any known PCA prognostic factor. No association between SUα1 and SUα3 expression levels and recurrence or death was found. SUα3:SUα1 ratio was significantly associated with PCA biochemical recurrence (adjusted Cox regression model, hazard ratio = 1.68, 95% confidence interval [1.03; 2.74]).
Conclusions: The SUα3:SUα1 ratio appears as an interesting potential PCA marker. Since Na^+/K^+ ATPase is the target of cardiotonic steroids, this marker might have important therapeutic implications.

Src/ezrin Pathway: A Novel Marker For Metastasis In Breast Cancer

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Background: The survival benefits of existing adjuvant therapies for breast cancer have been fully exploited; still, the identification of individuals that will relapse or have metastatic disease remains difficult. *Ezrin*, is a membrane-cytoskeleton crosslinker protein required for motility and survival of normal epithelial cells. It is frequently over-expressed in human breast cancer and is also required for metastasis. The mechanism by which ezrin is activated in metastatic disease is not known clearly. We recently showed that ezrin acts co-operatively with the non-receptor tyrosine kinase, Src, in cancer cells via phosphorylation of specific tyrosine residues. This study examines the role of Src/ezrin in metastatic breast cancer using a xenograft model.
Methodology: Metastatic breast cancer cells (AC2M2) expressing either *vector alone, or a Y477 ezrin mutant* (not phosphorylated by Src) were engrafted into the mammary fat pad of immunosuppressed mice. Primary tumours were excised at 21 days and the animals were sacrificed 40 days after engraftment.
Results: *Vector alone* tumours rapidly infiltrated into the surrounding stroma and abdominal muscle, and seeded onto visceral organs. The *Y477F ezrin mutant* tumours were well-circumscribed with no infiltration into the adjacent soft tissue. Lymphovascular invasion was evident in vector alone group only. Ezrin showed strong cytoplasmic staining in both the primary and metastatic nodules in vector alone group. There was both partial membranous and cytoplasmic staining in the *Y477F ezrin mutant* group.
Conclusions: Our study implicates a novel role of the Src/ezrin pathway in regulating local invasion and metastasis of breast cancer. We are in the process of correlating this interaction with outcome data using human breast cancer tissue microarray.

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A Case of Endocervical Adenocarcinoma with Metastasis to the Ovary

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The majority of endocervical adenocarcinomas are related to high-risk human papillomavirus (HPV). A recognized and rare phenomenon is the ability of minimally invasive and even in situ endocervical adenocarcinoma to metastasize to the ovaries, causing a diagnostic challenge. Fortunately, recent research has indicated that immunohistochemical staining with p16 can be a useful tool in addressing the likelihood that an ovarian tumour is metastatic from the cervix. We describe a 43-year-old female who was treated for a cervical adenocarcinoma in situ (AIS) in 2004. She presented five years later with recurrent disease in the cervix and a coincident 20 cm right ovarian neoplasm. Microscopy of the ovary revealed morphology suggestive of a primary borderline mucinous tumour of intestinal type. Microscopy of the cervix showed a proliferation of atypical glandular epithelium with some areas of intestinal-type differentiation, consistent with at least AIS. The histologic similarities between the cervical and ovarian neoplasms raised the possibility of metastatic disease in the ovary. Immunohistochemistry performed on the cervical lesion showed strong positivity for p16, mild CEA staining, and no staining for estrogen receptor or vimentin, consistent with an endocervical origin. Immunohistochemistry performed on the ovarian tumour revealed positive staining for cytokeratin 7 and p16, weak staining for CEA, and no significant staining for cytokeratin 20, estrogen receptor, vimentin, or CDX2. Given the clinical history, histology, and immunohistochemistry, we concluded that the ovarian neoplasm was consistent with a metastasis from the endocervical lesion. Further molecular diagnostic HPV testing on the cervical and ovarian lesions confirmed the presence of identical high risk HPV sub-types in both tissues. Recognizing that minimal endocervical disease can metastasize to the ovary, albeit rarely, is important and the application of p16 is particularly useful.

Histopathologic Characterization of Cervical Adenosquamous Carcinoma

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Background: Adenosquamous carcinoma of the cervix is defined as tumour composed of malignant glandular and squamous components that can be appreciated without special stains. It has been suggested that it originates from biphasic differentiation of reserve cells. We aim to characterize their histopathologic features emphasizing the proportion of the histologic components and its precursor lesion.

Design: Cases accessioned between 1999 and 2009 were retrieved from the archive of Anatomic Pathology. Two gynecologic pathologists reviewed all available slides and classified them according to the proportion of histologic components into predominantly squamous, predominantly glandular or indeterminate. Associated in situ lesions were classified into squamous carcinoma in situ (SCIS), or adenocarcinoma in situ (AIS) and its subtypes. Fisher's exact test was used to determine differences between the groups.

Results: Fifty-five cases met the WHO criteria for the diagnosis of adenosquamous carcinoma, including 32 hysterectomies, 7 radical trachelectomies, 3 cone/LEEP and 13 cervical biopsies. At diagnosis, the median patient age was 44 years (range: 28-79); 43 Stage I, 5 Stage II and 7 Stage III. More cases showed a predominant squamous component (35 vs. 17 with a predominant glandular and 3 were indeterminate). Only in 7/55 (12.7%) cases the squamous component was of keratinizing type. An in situ component was identified in 39 (70.9%) cases: 18 of those were SCIS, 9 AIS-alone and 12 SCIS+AIS. All AIS were of endocervical type except 3- adenosquamous and 1-intestinal type. AIS was significantly associated with predominantly glandular component ($p = .0019$) and SCIS was significantly associated with predominantly squamous component ($p = .035$).
Lymphovascular invasion (LVI) was seen in 27/55 (49.1%) of the cases. In 7/38 cases the lymph nodes were positive for metastatic carcinoma: 4 cases exhibited squamous, 1 glandular and 2 both elements in metastases. LVI and nodal

metastases were not significantly associated with the predominant component histology (p = .13 and 0.67 respectively).

Conclusion: Adenosquamous carcinoma exhibits variation in the proportion of squamous and glandular components in both invasive and in situ elements. The predominant component of the invasive element correlates with the precursor lesion but not with lymph node metastases or LVI.

Non Small Cell Neuroendocrine Carcinoma of the Uterus: Clinicopathologic Features of Seven Cases

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Background: Non-small cell neuroendocrine carcinomas of the uterus are rare tumours that can be pure, combined with endometrioid carcinoma, or a component of malignant mixed mullerian tumour (MMMT). Many studies reported that neuroendocrine differentiation is a marker for aggressiveness. In the female genital tract, primary tumours with neuroendocrine features are rare and involve uterine cervix most commonly. We aimed to study the clinicopathologic features of this rare tumour.

Design: The clinicopathologic features and follow up of 7 cases of hysterectomy specimens with the diagnosis of non small cell neuroendocrine carcinoma of uterus, retrieved from the archive of the Department of pathology at our institution between 2003-2009, were reported. The presence of an associated adenocarcinomatous component was noted. No additional immunostaining was performed.

Result: Patients with average age 64 years (range 45-84 years) presented with postmenopausal bleeding. 2 patients had bone metastases at presentation. No one presented endocrine syndrome. 4 patients had stage IA tumours, 3 had stage IIIA. The follow-up period was 1-80 months. The neuroendocrine component with insular, solid or pseudorosettes pattern, was characterized by large cells with abundant mitotic figures and foci of necrosis. This component had a multifocal to diffuse positivity for at least one neuroendocrine marker (Chromogranin, synaptophysin) with a cytokeratine dot-like positivity (EMA, PanCK). Five tumours were composite tumours with a dominant component of neuroendocrine carcinoma (90%) and a component of well differentiated endometroid carcinoma (3 tumours) or of poorly differentiated endometroid carcinoma (2 tumours); 1 was pure neuroendocrine carcinoma and 1 presented a MMMT with a neuroendocrine component. All patients were treated by TAH, BSO and pelvic lymphadenectomy, followed by radiation therapy and/or chemotherapy except in 1 patient dead within 1 month of the surgery. No evidence of progression was noted at 31-80 months follow-up in 4/7 patients. Patients dead (2) with the disease were stage III and presented with metastasis with follow up (1-16 months).

Conclusion: We reported 7 cases of non small cell neuroendocrine carcinoma of the uterus, pure or combined with endometroid carcinoma or MMMT. Immunostaining for neuroendocrine markers was required for diagnosis. Postoperative adjuvant therapy had a positive impact on the long-term survival.

Octaplex Halifax Experience

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Purpose: Octaplex is a plasma derived product that was first introduced in Canada in 2008. Our study aimed to monitor the experience at our academic teaching hospital using this product. In addition, we tested the efficiency of the standard dose recommended.

Method: Charts of all patients receiving Octaplex from February to end of December 2009 were retrospectively reviewed to collect data.

Results: During the study period, a total of 50 patients received Octaplex; 38 of which met the criteria (I: group) set by the National Advisory Committee on Blood and Blood Products (NAC). Of those, 16 patients presented with

bleeding (IB) and 22 received it for undergoing invasive procedures (IP). The mean INR was 3.9 pre –octaplex and 1.6 post-octaplex in the (IB) group; in (IP) group, it was (3.3) pre- and (1.6) post-octaplex. When the INR was between 2 and 4, in (I) group, the first dose of octaplex, brought it down to a mean of 1.67, when INR was between 4 and 6, it was brought down to a mean of 1.61, and when INR was more than 6, it went down to a mean of 2.2. Moreover, 69% of patients with INR (2-4) had post-octaplex INR of 1.7 or less and 85% of patients with INR (4-6) had of INR 1.7 or less. More importantly, the initial recommended dose was enough to stop the bleeding in all patients.

Conclusions: Our data showed that patients presenting with pre-octaplex INR level of less than 6, will likely not require more than our standard initial dose of 40 mL (1000 IU of factor IX) that is recommended by NAC. Following this standard dose, bed side monitoring for bleeding and post-octaplex INR would determine if there is need for an additional dose.

Title: Radiological, Cytological and Histological Correlation in Bronchoalveolar Carcinoma

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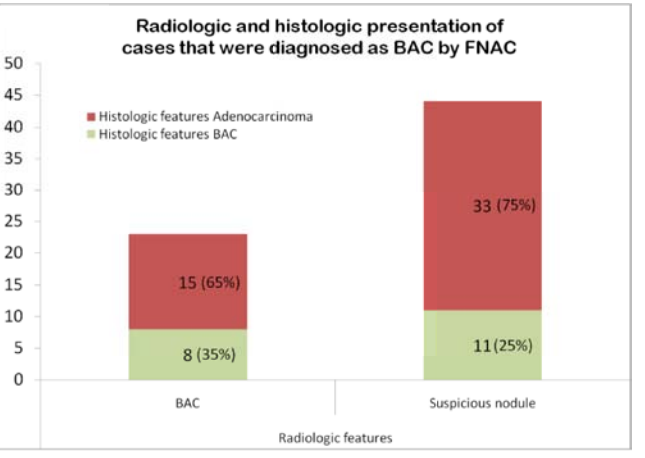
Background: Bronchoalveolar carcinoma (BAC) is considered to be an in situ lesion, and surgical resection is assumed to be curative. It presents as a space occupying lesion in the lungs, appearing as a ground glass opacity which is then biopsied for cytological evaluation. Demonstration of a well-differentiated, localized tumour with lepidic growth pattern suggests a BAC. But well differentiated adenocarcinoma or an adenocarcinoma of mixed subtype with invasive patterns can also give a similar deceiving picture. The rate of concordance of radiologic and cytologic appearance of BAC with the subsequent final histological diagnosis is not well established.

Design: We did a retrospective search of all cases that were diagnosed as 'BAC' following FNAC at the Ottawa Hospital (00-09). All cases had a prior CT scan and needed to have a subsequent wedge resection or lobectomy with evaluations done at our institution. Correlations between radiologic, cytological and subsequent histopathological diagnosis was then evaluated.

Result: 71 cases diagnosed as BAC by FNAC were found that had a prior CT scan and a subsequent surgical resection. Among these only 23 (32%) cases had a distinct ground glass opacity on CT scan. 8 (35%) of these were subsequently confirmed as BAC. 44(62%) cases did not have the radiologic appearance of BAC, but 11 (25%) of these were subsequently found to be BAC. Altogether only 28 cases (37%) were confirmed as BAC, 26 (36%) were invasive adenocarcinoma, 14 (19%) had a mixed subtype of both BAC and adenocarcinoma, and the remaining (5%) had multiple other malignant diagnoses.

Conclusion: The study shows that only 35% of lesions appearing as BAC on CT scan are subsequently confirmed by both cytology and histology. A cytologic diagnosis of BAC is inaccurate in >60% of cases. Such misdiagnosis of an invasive adenocarcinoma as BAC may give a wrong sense of relief to the clinician and patient, and even delay treatment. Since BAC is mostly a diagnosis of exclusion that cannot be made without histologic evaluation, we propose that, 'BAC' diagnosis should not be made by "radiology" or "cytology" alone, but rather needs a combined clinical, radiological and histo-pathological evaluation. Further studies are required to identify reasons for the discrepancy between radiological, cytological and histopathological diagnosis of BAC.



Pituitary Necrotizing Granulomas Presenting as a Sellar Mass

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We report a case of a 45-year-old Colombian female who presented with 3-months history of headache anorexia and fatigue with diplopia in addition to left facial nerve palsy 2 weeks prior to presentation. On examination the visual fields and fundus were normal and left sixth cranial and facial nerve palsies were noted. Chest radiograpy was normal. Serum prolactin was 38.5ng/mL (1-25), TSH 0.086UI/ml (0.4-4) and free T4 was 4.75mg/dL (4.5-12). MRI disclosed a sellar mass with suprasellar but no parasellar or retrosellar extension. The lesion interpreted as a pituitary tumour, was resected through a transsphenoidal approach. Histopathological examination of the excised tissue revealed caseating granulomas inside the pituitary. The necrotic areas were surrounded by epitheloid macrophages, lymphocytes and a few multinucleated giant cells. In the remaining adenohypophysis immuno-histochemistry demonstrated the presence of various adenohypophyseal cell types. Staining for acid fast bacilli and fungi was negative. It is difficult to prove the etiology of caseating granulomas in the pituitary. The differential diagnosis includes tuberculosis (despite no prior history), sarcoidosis, syphilis, mycotic granuloma, autoimmune granulomatous hypophysitis, histiocytosis X and Erdheim Chester disease. Post-operative antituberculous medication resulted in resolution of the sixth and seventh cranial nerve palsies within one month.

Hepatic Metastasis of Composite Adenocarcinoma and Neuroendocrine Carcinoma

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Background: Composite adenocarcinoma and neuro-endocrine carcinomas of the GI tract are rare. We describe two cases of composite adenocarcinoma and neuro-endocrine carcinoma including the first case report of a colonic adenocarcinoma and neuroendocrine carcinoma diagnosed following hepatic wedge resections for metastatic disease

Case presentation: Case 1: A 58-year-old female with cecal cancer was managed by right hemicolectomy. Surgical pathology diagnosed mucinous colonic carcinoma with transmural invasion into the visceral peritoneum. Surveillance CT showed a predominant lesion in segment 8 of the liver and two smaller hypoattenuated lesions in segment 4. Laparotomy with intraoperative ultrasound verified the presence of three potential metastatic foci, with no other focal liver lesions. Pathological examination showed three lesions with features of composite adenocarcinoma and neuroendocrine carcinoma. Case 2: A 62-year-old male with clinical history of obstructive jaundice and periampullary mass was managed by Whipple's procedure and cholecystectomy. Surgical pathology revealed a neuroendocrine carcinoma combined with mucin secreting adenocarcinoma of the ampulla of Vater and distal common bile duct

Conclusion: We present two cases of composite adeno-neuroendocrine carcinoma of the GI tract including an unusual case of metastatic composite tumour to liver initially diagnosed as mucinous adenocarcinoma of colon. Review of the colonic carcinoma showed mucinous adenocarcinoma with scattered small nests of neuroendocrine cells present. The true nature of the tumour became more evident in the metastatic liver lesion.

Cytohistologic Correlation of Atypical Squamous Cells, Cannot Exclude a High Grade Squamous Intraepithelial Lesion (Asc-H)

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Introduction: A diagnosis of ASC-H is made in approximately 0.4% of all Pap tests, with colposcopy recommended in these cases. The associated risk of having a high grade lesion (CIN II or greater) on follow-up cervical biopsy is variable, and ranges from 10-68%. Some negative biopsies are expected, since ASC-H represents true dysplasia as well as its mimics (including age-related atrophy). We undertook an ASC-H cytohistologic correlation study at our institution.

Methods: After research ethics board approval, we included all 377 patients with diagnosis of ASC-H on conventional Pap tests in 2008. Age and subsequent histology, if available, were recorded for each case.

Results: For all patients, mean age was 34.3 years. Sixty-one patients (16%) had a negative first biopsy (mean age 37.9), 75 (20%) had CIN I (mean age 29.9), 141 (37%) had CIN II-III (mean age 32.1), and 5 (1.3%) patients had an invasive carcinoma (mean age 53.6). The remainder had no available histology. On initial biopsy, the positive predictive value of ASC-H for high grade dysplasia was 50.9%. Patients with negative biopsies and those with invasive carcinoma were significantly older than those with CIN I and CIN II-III. There was no significant age difference between the CIN I and the CIN II-III groups. Of the 61 patients with an initial negative biopsy, 14 (23%) had CIN II or greater on a second or third cervical biopsy.

Conclusions: At our institution, the rate of ASC-H diagnosis and associated correlation with high-grade dysplasia is comparable to the published literature. Patients with invasive carcinoma and negative biopsies were older than those with low and high grade dysplasia. A significant portion of patients with negative initial histology had high grade dysplasia on further biopsy, warranting close follow-up in these patients.

Quantitation of HER-2/*neu* Protein Expression by Image-Analysis Using ImageJ: Comparison of Different Methodologies

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HER-2/*neu* is a cellular membrane protein often targeted for breast cancer therapy. Image-analysis of HER2/*neu* immunohistochemical staining is considered a viable alternative to manual scoring in order to enhance reproducibility and reliability. Image–analysis quantitation of this signal is usually performed using a "skeleton-like" mask which tries to assess only the cellular membrane area. Because tissue sections generally have a thickness of 4-6 microns, cellular membranes do not occupy a two-dimensional space within the section. Sixty (60) serial sections from the same Her2/*neu* positive control tissue block were stained with the A0485 polyclonal antibody (DakoCytomation, Carpinteria, CA, USA). They were quantitated using the standard membrane-based "skeleton-like" mask (SLM) and masks derived from the signal intensity (SIM). The latter integrates the 3-dimensional features of the signal. An open source software (ImageJ) was used for this study. The SIM mask showed a smaller coefficient of variation compared to the SLM. We also correlated normalized computer-derived IHC results obtained using both SIM and SLM in 500 breast cancer cases for which HER2 status was determined using CAP/ASCO guidelines. Both approaches discriminate similarly amplified and non-amplified tumours. SIM offers a reliable and simpler method for HER2/*neu* IHC quantitation.

Osteosarcoma Arising in Cemento-Ossifying Fibroma

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Cemento-ossifying fibroma (COF) of the jaw is a benign

fibro-osseous lesion. We report a case of an osteosarcoma arising in a COF in a 21-year-old female. She presented five-years previously with a gradually enlarging, radiographically well defined, mixed lytic/blastic lesion of her right mandible. Biopsy showed a benign fibro-osseous lesion consistent with COF. Definitive excision was not undertaken. The lesion was stable until four-years later, at which point rapid growth occurred and a subsequent biopsy showed a fibro-osseous lesion with increased atypia and osseous matrix deposition when compared to the original biopsy of the COF. The lesion was resected en bloc. Histological examination revealed residual cemento-ossifying fibroma with transition to areas of conventional osteoblastic osteosarcoma. A review of published literature reveals no cases of malignant transformation of a COF. This case represents a very rare phenomenon that has not, to the best of our knowledge, ever been reported.

Conclusion: This case demonstrates that an osteosarcoma can arise, albeit rarely, in an ossifying fibroma and that close clinical follow-up is warranted.

Carcinoma Cuniculatum of the Penis: A Rare Variant of Squamous Cell Carcinoma

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Carcinoma cuniculatum of the penis is an exceptionally rare variant of squamous cell carcinoma. It is characterized by a deeply endophytic branching and burrowing growth pattern that simulates rabbit burrows. It may occur throughout the body, though only one documented case series describes the lesion occurring in the penis. Clinically it displays hybrid features of invasive squamous cell carcinoma and verrucous carcinoma; it has jagged infiltrative edges and may invade deeply into the corporal tissues, but metastases have not been described. Thus, the treatment intent is curative even in deeply infiltrating lesions. Herein we report a case of a 55-year-old with disease located on the ventral aspect of the shaft of the penis. He subsequently underwent a partial penectomy. Although the histologic features were characteristic, the tumour invaded only into the deep dermal connective tissue, comparatively superficial to all previous documented cases. The case is discussed and the verruciform penile carcinomas are reviewed.

Solitary Fibrous Tumour of Urinary Bladder: Report of 3 Cases and Review of Literature

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Solitary fibrous tumour (SFT) as thought earlier is not site specific. We report three cases involving urinary bladder. One of the patients diagnosed by cystoscopic biopsy subsequently underwent cystoprostectomy because of large mass. All three tumours are benign by histological criteria.

100% Rapid Pre-Screening of Pap Smears: An Efficient Quality Control Technique to Detect False-Negative Cases

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Despite being commonly used, the 10% random re-screening of negative Pap smears is known to be very inefficient to detect false-negative (FN) cases. In contrast, the lesser known rapid pre-screening (RPS) technique of 100% Pap smears has been shown to be much more efficient to detect FN cases in gynecologic cytology; for this reason we replaced the random 10% re-screening by RPS in our laboratory July 2004.

Objective: We report on the impact of RPS technique on our laboratory's performance, in particular on FN rate and on monitoring of cytotechnologists' (CT) screening sensitivity, in a recent 8-month period.

Methods: All routine conventional Pap smears (n = 22,287) over 8 months underwent RPS by 12 cytotechnologists

(high-risk cases were excluded). Approximately 45 seconds were allowed to rapidly pre-screen each slide. The presence of abnormal cells (ASCUS or above) detected on RPS was documented. All slides subsequently underwent routine full screening. Results of both screening methods were compared for each CT and for the overall laboratory.

Results: The RPS ASCUS+ sensitivity for the entire laboratory was 46.7% (range for CTs = 25–73.2%) while the ASCUS+ full screening sensitivity for the overall laboratory was 92.6% (range for CTs = 84–100%). Thirty-five (35) FN cases were detected by RPS (24 ASCUS, 1 ASC-H, 8 LSIL, 1 HSIL, 1 adenocarcinoma). The overall laboratory FN rate during these 8 months was 7.4% (or 14.8% if corrected for the RPS sensitivity).

Conclusion: RPS is an efficient technique to detect FN cases and to monitor sensitivity in gynecologic cytology.

Extranodal Rosai-Dorfman Disease Involving Colon, a Rare Presentation: A Case Report with Review of the Literature

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Rosai-Dorfman disease (RDD) is a rare, idiopathic and non-neoplastic histiocytic disorder. The most frequent presenting symptom is painless cervical lymphadenopathy in young patients. However, in about 25% of cases there is no lymph node involvement, and the disease presents as a mass in skin, soft tissue, nasal cavity, eye, bone, or other extranodal sites (extranodal type). This can pose a diagnostic challenge if RDD is not considered in differential diagnoses. Gastrointestinal (GI) involvement of RDD is extremely rare; only 14 cases had been reported.

We report a case of extranodal RDD involving the GI tract in a 24-year-old African-American woman. She presented with lower abdominal pain, constipation and mild back pain for two years. MRI revealed an 8 cm mass present in her pelvic cavity. During surgery, an irregular colonic mass was found to adhere extensively to pelvic organs. Because the mass could not be completely removed, a partial resection of the mass with attached segment of colon was done. Microscopically, the mass was well demarcated, partially covered with serosa, extended into the muscularis propria and submucosa of the colon. It was composed of predominantly spindle-shaped or epithelioid histiocytes, intermixed with plasma cells and lymphocytes. These histiocytes have abundant pale eosinophilic cytoplasm, well defined delicate nuclear membrane, enlarged round or oval vesicular nuclei, and single prominent nucleolus. Rare histiocytes showed emperipolesis. The colonic mucosa was intact. Three lymph nodes were identified, which were not involved by RDD. Immunohistochemically, the histiocytes were positive for S100 (strong) and CD68, and negative for c-kit and CD1a. The above morphological and immunohistochemical features are diagnostic for RDD, extranodal type.

Intracystic Papillary Carcinoma in Male Breast Metastatic to Lymph Node

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Intracystic (encapsulated) papillary carcinoma (IPC) of the breast is an indolent tumour that rarely invades locally or metastasizes to lymph nodes and is extremely rare in males. The few literature reports of male IPC to date have not described any case with lymph node involvement. We report the case of invasive IPC in a 61-year-old man with nodal metastasis. The patient presented with a self-identified left breast mass, which on core biopsy showed invasive ductal carcinoma (IDC), no special type, arising on a background of IPC. He then underwent total mastectomy with axillary dissection. A 2.2 cm retroareolar, solid mass with a peripheral cystic area was visible on the gross specimen. Microscopically, the cystic area contained atypical cells with a compact papillary growth pattern lined by a thick fibrous capsule. Immunostaining demonstrated an absence of myoepithelial cells within the atypical cells or lining the cyst, thus confirming the presence of IPC. Moreover, an invasive proliferation of cells arising from the periphery of the cyst also stained negative for myoepithelial markers and was diagnosed as IDC arising from IPC. One of 8 lymph nodes

in the axillary dissection contained a 2.4 cm metastasis with extranodal extension. Interestingly, the metastasis (surrounded by a rim of lymphoid tissue) reiterated the cystic architecture of the primary, containing both IPC and IDC components. Both the primary and metastatic tumours were estrogen and progesterone receptor positive and Her2/neu negative. This case describes the previously unreported finding of male breast IPC with lymph node macrometastasis in a tumour that seldom involves lymph nodes even in females.

Ovarian Adenosarcoma Arising from Benign Cystadenoma and Associated Intraoperative Consultation Pitfalls

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Adenosarcoma of the ovary arising in the background of serous cystadenoma is an extremely rare presentation of an uncommon diagnosis that can lead to diagnostic errors during intraoperative consultation (IOC). An unusual case of ovarian adenosarcoma arising from a smooth-walled serous cystadenoma is described. A 61-year-old woman was found to have incidental bilateral ovarian cysts on routine abdominal ultrasound and underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy and omentectomy with IOC requested on both ovaries. The right ovary was replaced by a multiloculated, fluid-filled cyst without any solid or papillary areas. The malignant component was under-diagnosed during frozen section as benign cystadenoma due to the deceptively benign gross appearance of the tumour. On permanent sections, a phyllodes-like pattern of stromal proliferation and periglandular condensation of atypical stromal cells with a mitotic count of 3 per 10 HPF was more apparent and led to the diagnosis of adenosarcoma. Immunohistochemistry was unable to distinguish the malignant component from the benign. To our knowledge, this is the first reported case of an adenosarcoma arising from a grossly benign cystadenoma and the third case in the literature of an adenosarcoma associated with a cystadeno(fibro)ma. This case also illustrates the challenges in differentiating adenosarcoma from a benign counterpart on frozen and permanent sections.

Keywords: adenosarcoma, cystadenoma, frozen section, intraoperative consultation, ovary.

Follicular Lymphoma In Situ of Spleen and Lymph Nodes: Diagnosis and Implications

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Follicular lymphoma (FL) accounts for about 30% of non-Hodgkin lymphomas, and is most often detected at an advanced stage. However, architecturally benign-appearing lymph nodes with one or more follicles displaying features otherwise typical of FL, including expression of bcl-2, have been described recently. This process is considered to represent intra-follicular neoplasia/*in-situ* follicular lymphoma, and is included in the 2008 edition of the 'WHO Classification.' We present herein a series of three such cases, one in the spleen and two in lymph nodes, in which *in-situ* FL was diagnosed at initial presentation. Careful examination of all three cases revealed a monotonous proliferation of centrocytes in a few germinal centers with co-expression of bcl-2, bcl-6 and CD10, within otherwise architecturally intact lymphoid organs. After appropriate investigation, the patients are being followed without therapy. There have only been limited reports of cases of *in-situ* FL in lymph nodes and one report of this finding in other lymphoid organs. We review the current literature on the topic and propose the term "follicular lymphoma *in-situ*" (FLIS) as an alternate designation to conform to standard nomenclature frequently utilized for epithelial malignancies. The clinical significance of this diagnosis and its therapy remain to be clarified. Finally, consideration should be given to routine immunostaining of apparently reactive lymph nodes for its improved detection.

Herpes Simplex Virus 2 Herpetic Hepatits in an Immunocompetent Patient: A Case Report

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Herpetic hepatitis is a rare and usually fatal disorder, with fewer than 100 cases reported in the literature, predominantly in immunocompetent people. We report the case of a 64-year-old woman with no prior history of immunodeficiency who died of herpetic hepatitis first diagnosed at autopsy. Her past medical history was significant for epilepsy, myocardial infarction and a recent diagnosis of celiac disease. She presented to the emergency department with weakness and malnutrition. She progressively deteriorated over three weeks in hospital with a decreasing level of consciousness and disseminated intravascular coagulation. She was transferred to the intensive care unit due to her level of consciousness and experienced just two days of significantly elevated liver enzymes before her death. Clinically, her death was attributed to celiac disease. As is often the case, herpetic hepatitis was not suspected prior to her death. On gross examination her liver was pale but otherwise unremarkable; no mucocutaneous lesions were noted. Microscopic examination of her liver showed extensive necrosis and herpetic inclusions; immunohistochemistry was positive for herpes simplex virus 2 (HSV2). Post-mortem HIV assays were negative. Only seven cases of HSV2 herpetic hepatitis in non-pregnant immunocompetent patients have been reported. Her celiac disease and related malnutrition may have contributed to diminished immunity, possibly rendering her susceptible to this rare disease. A full review of the pathologic findings and of herpetic hepatitis will be presented.

The Experience of Dual-Colour Double Immuno-histochemistry Studies in Rimouski-Neigette

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Over the years, the number of IHC studies has been increasing in several laboratories. This is especially true in the laboratory of the CSSS of Rimouski-Neigette where dual-colour immunohistochemistry has been used since 1999. However, the advent of Polymeric Detection Kits that allow double colorations during the same cycle can maximize the number of IHC studies done during each cycle.

Since 1999 we have developed several protocols of dual-colour IHC with winning combinations of antibodies. Our experience has shown that this technique offers several benefits over single IHC by: (1) reducing time needed for slide preparations; (2) reducing use of controls; (3) saving costs on ultra resistant labels and antigen retrieval reagents; (4) increasing the number of IHC studies that can be performed during a cycle; (5) carrying out more IHC studies on scanty pieces of tissue. Dual-colour IHC also offers the pathologist several diagnostic benefits by: (1) demonstrating double antigen positivity within the same cells; (2) demonstrating lymphocytes/plasma cells clonality (lambda/kappa) even focal; (3) allowing rapid evaluation of the myeloid/erythroid ratio (MPO/HbA) in bone marrow biopsies; (4) reducing the time for the interpretation of IHC by decreasing the number of slides. Drawbacks of the technique include: (1) longer staining cycles; (2) a weak staining by an antibody can be hidden by a strong staining of the other; (3) an aberrant staining by one antibody could be hidden by the staining of the other; (4) the brown color contains a little tinge of red and some pathologists may find it difficult to distinguish it from red. In light of our experience, we believe that dual-color IHC studies are an interesting addition to the arsenal of diagnostic techniques used in surgical pathology laboratories. They represent an improvement that could be used according to specific needs of pathologists.

A Systematic Review and Critical Assessment of the Correlation of Circulating Malignant Cells and Disease Outcome in Cutaneous Melanoma: How Can We Improve?

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Our objective was to review current assessments of the prognostic significance of circulating malignant cells

(CMCs) in cutaneous metastatic melanoma and to suggest a standardized protocol for future investigations. We included all humans studies assessing the prognostic value of melanoma markers in the last 10 years. The restriction criteria excluded studies with <20 patients enrolled, uveal and other melanomas, studies limited to nodal or bone marrow RT-PCR, studies evaluating non-specific melanoma markers and studies assessing the quality of the technique. Using these criteria, 28 original articles and 3 meta-analyses were evaluated. Significant heterogeneity of the methods was found including differences in patient selection, type and number of tumour marker analysed, amount of blood sample, methods of CMC detection and stage of disease. The PCR positivity rates for cutaneous melanoma were extremely variable, ranging from 20% to 82% for tyrosinase (TYR). Studies with longer follow up and using multiple markers were more conclusive in identifying a prognostic correlation between the presence of CMCs and overall survival. In conclusion, a standardized protocol involving quantitative real time PCR with normalized cut-off values and multiple samples will provide us with the ideal methodology to predict disease outcome.

Can Cervical Adenosarcomas be Treated Conservatively?

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Objective: Cervical adenosarcomas (AS) are rare tumours for which pathological data and treatment experience are limited. In the current study, we analyzed their histological features as putative indicators for depth of invasion and residual disease (RD) in hysterectomy specimens to assess the amenability of conservative surgical treatment for young patients.

Method: Cases of cervical AS accessioned between 2000 and 2009 were retrieved from our archives. Slides and charts were reviewed and the following data was recorded: initial biopsy (bx) or polypectomy (px), tumor size, multifocality, mitotic rate, sarcomatous overgrowth (SO), depth of invasion and RD in the hysterectomy.

Results: We identified 8 cases of cervical AS in patients aged from 33 to 64 years (5 patients were under the age of 50). All patients underwent hysterectomy, which showed no RD in 3 cases (37.5%). In the 5 cases with RD, 1 had no cervical wall invasion, 3 showed invasion limited to the inner third (maximal depth: 5mm) and only 1 showed invasion into the middle third (depth: 8mm). This latter patient had other ominous features (mitotic index of 100/10HPFs, multifocal disease, tumour size of 10 cm, SO) and was the only one who experienced recurrence. On follow up (1–67 months) all other patients were free of disease. Patients with no RD had smaller tumours (1.1–2.2 cm vs. 4.5–10 cm), lower mitotic rate (1-3/10HPFs vs. 3-100/10HPFs), were more initially sampled by px (3/3 vs. 2/5) and they did not show multifocal disease (0/3 vs. 3/5) or SO (0/3 vs. 1/5). A tumour size < 4 cm significantly correlated with the likelihood of having no RD (Fisher, $p = 0.018$).

Conclusions: 62.5% of cervical AS had RD, but depth of invasion was minimal in all but 1 case. A tumour size < 4 cm significantly correlated with the likelihood of having no RD and therefore cervical AS measuring < 4 cm initially excised by px may be amenable to conservative treatment, such as cone biopsy.

Myopericytoma: A Pleural Based Spindle Cell Neoplasm Off the Beaten Path

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Background: Myopericytoma is a recently described hemangioperictyoma-like neoplasm with myoid differentiation. These tumours are typically located in the subcutaneous and soft tissues of the extremities. We report a novel pleural based pulmonary myopericytoma.

Design: A 58-year-old asymptomatic female was incidentally found to have a right upper lobe (RUL) pulmonary nodule on a chest radiograph. Her past medical history included a 30-pack-year history of cigarette smoking

and rheumatoid arthritis. Computed tomography (CT) showed a well-defined solitary pleural based 1.0 cm nodule which on core biopsy was diagnosed as a benign proliferation of uniform spindle cells. At the five year follow-up CT, the lesion had doubled in size, now measuring 2.7 × 2.0 cm. A core biopsy was repeated. The patient underwent a wedge resection of the RUL anterior segment.

Results: The subpleural lesion was grossly homogenous and well- circumscribed. Microscopically, it was composed of densely packed spindle cells organized as whorls and short interlacing fascicles with a concentric perivascular distribution. Immunohistochemical reactions were positive for vimentin, smooth muscle actin (SMA), muscle-specific actin (MSA) and Bcl-2 and negative for CD34, desmin, h-caldesmon and cytokeratin. Atypically, increased mitotic activity was noted (average 7/10 HPFs) but no other malignant features were identified. The final diagnosis was myopericytoma with atypical features, due to increased mitotic activity.

Discussion: Pulmonary myopericytomas are rare and a pleural based lesion is a novel finding. Conventional spindle cell lesions of the pleura had to be excluded to avoid misdiagnosis and potential mistreatment.

Composite Mantle cell and Follicular Lymphomas: A Rare Diagnostic Pitfall

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Composite low grade B-cell lymphomas containing two phenotypically distinct cell populations corresponding to separate B-cell non-Hodgkin lymphoma (NHL) entities representing a true collision tumour with two unrelated malignant clones, is an extremely rarely encountered diagnosis. More commonly though still rare is a divergent differentiation within a single neoplastic process, which represents a transformation, and should not be interpreted as a composite lymphoma.

We report a rare case of composite follicular lymphoma (FL) and mantle cell lymphoma (MCL), that may present a potential diagnostic pitfall leading to management implications. The distinction between MCL and other small B-cell NHLs is important because MCL has a more aggressive clinical course. In bone marrow (BM) biopsy specimens, this distinction can be particularly difficult.

I report a 76-year-old Canadian female, originally from Italy, presents with dyspepsia, and itching for several months prior to examination. Her symptoms are chronic and progressive but are not associated with fever, weight loss or night sweats. Her medical history is significant for hepatitis C (grade1/4 and stage1/4), osteoporosis, nephrolithiasis, patent foramen ovale depression and anxiety. Her current medications include mitrazepine, pantoprazole and citalopram. Physical examination reveals absence of lymphadenopathy and organomegaly. Her blood picture shows neutropenia (absolute neutrophilic count 0.3 × 10 /L), and mild left shift. Abdominal and pelvic computed tomography (CT) scan with contrast revealed lobulated hypo-attenuating mass arising from the superior portion of the spleen. It measured 9.0 × 8.0 cm. Periaortic and paraaortic lymphadenopathy is noted measuring 1.2 cm in maximum dimension. Chest CT scan shows mediastinal lymphadenopathy measuring 4.0 × 3.0 × 2.5 cm in maximum dimension with a 5.0 cm craniocaudal extension. CNS imaging demonstrates white matter foci compatible with incidental small vessel ischemia but no other discernible. An excisional lymph node biopsy is performed through mediastinoscopy.

Gross examination showed multiple tissue fragments measuring 4 × 4 cm in aggregates that were submitted as per lymphoma protocol.

Microscopic sections showed predominantly nodular (90%) and diffuse (10%) pattern with mixed small and occasional large cells with irregular nuclei. Residual mantle cell cuff is noted focally. Immunohistochemistry revealed expression of CD10, CD20, and Bcl-6 but negative staining for Bcl-2 and CD5 in the centers of the nodules. The periphery of the nodules showed positive staining for CD5, CD20, *bcl-2* and cyclin D-1 but negative staining for CD3 and CD10. The Ki 67 proliferation index is about 15% in the center of the follicles in contrast to the thickened peripheral mantles of the follicles that has a Ki67 proliferation index of 30%

Flow cytometry assay of the mediastinal lymph node biopsy showed lymphocytosis, predominantly B lymphocytes (70%) with two distinct monoclonal populations. The first population accounts for 50% of CD19 and CD20 positive cells and consists of medium sized cells co-expressing CD10,

FMC7, and CD23, but are negative to CD5, and CD43. The second population is formed of small cells accounting for 30% of CD19 and CD20 positive cells and co-expressing CD5, and CD43. They are negative to CD10, CD23, and FMC7. Both populations were kappa restricted. Quantitative real-time polymerase chain reaction (PCR) confirms the presence of composite lymphoma with presence of *bcl-2* t(14;18) and bcl-1 t(11;14) translocations.

A diagnosis of composite follicular lymphoma low grade (2/3) and mantle cell lymphoma is made. The patient received chemotherapy using CVP-rituximab. Follow up for a year shows that the patient is alive.

Recognition of the mantle cell component in the current case is crucial as a single agent as rituximab cannot cure MCL, in contrast to FL. To the best to our knowledge, composite MCL and FL has been rarely reported previously in the English literature with only a single case report present.

The Clinical Significance of Renal Cell Carcinoma With Florid Giant Cell Component, Case Report and Review of the Literature

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Background: Renal cell carcinoma with multinucleated giant cells has been reported in the literature. Different types of multinucleated giant cells have been described. The syncytial multinucleated giant cells are rare and only few cases are reported. We present the first report of clear cell RCC with extensive giant cell component.

Design: We describe a unique case of a high grade clear cell RCC with its morphologic and IHC characteristics and review the published literature of similar cases.

Results: A 58-years-old man with a large right sided kidney mass and a 4 cm pre-aortic node. A right radical nephrectomy was performed. The nephrectomy specimen contained a 13.5 cm mass that extended into the peri-nephric tissue. Microscopically the tumour is formed of high grade clear cell RCC with conventional clear cell component and a second component showing rhabdoid features. More than 50% of the tumour was formed of bizarre syncytial giant cells in a nested pattern. The giant cell component stained positive for CD10, RCC, vimentin, and was negative for EMA, pancytokeratin, desmin and CD68. No other classic sarcomatoid component with spindle cells was identified.

Conclusion: Different types of giant cells have been reported in RCC. Their significance is unknown. Giant cells in RCC might represent either fused macrophages or mononuclear tumour cells. The clinical implications of giant cells vary according to their nature. The identity of the giant cells has to be investigated because of their potential difference in survival.

Patterns of Smoothelin Expression In Uterine Smooth Muscle and Smooth Muscle Tumours of the Uterus

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Background: Smoothelin is a novel smooth muscle-specific marker expressed only in terminally differentiated smooth muscle cells as part of its contractile cytoskeleton. Smoothelin expression is absent or decreased in noncontractile and proliferative smooth muscle cells. The purpose of this study is to evaluate the pattern of smoothelin protein expression in smooth muscle tumours of the uterus using immunohistochemistry.

Design: Seven cases of leiomyoma, six cases of cellular leiomyoma, three cases of atypical leiomyoma and five cases of leiomyosarcoma were identified from the St. Michael's Hospital pathology department database. Immuno-histochemical staining for smoothelin was performed according to the published protocol. The staining pattern, cytoplasmic or nuclear, was recorded. The staining was also scored as follows: 0 (less than 5% of cells staining), +1 (focal, 5-10% of cells staining, +2 (moderate, 11% to 50% of cells

staining), and +3 or (strong, diffuse staining in >50% of cells).

Results: Normal uterine smooth muscle showed a +3 strong, diffuse cytoplasmic pattern of Smoothelin staining. Leiomyomas showed a weak cytoplasmic staining pattern (+1) in 6/7 cases, while cellular leiomyomas showed very minimal and focal weak staining (0 score, < 5 % of the muscle fibres were staining) in 5/6 cases. 1/3 of the atypical leiomyomas showed +1 focal staining, with both a nuclear and cytoplasmic pattern. The leiomyosarcomas, all showed nuclear staining: 3/5 cases scored +1, 1/5 cases scored +2 and 1/5 scored +3.

Conclusions: Immunohistochemical expression of smoothelin is markedly decreased in leiomyomas of the uterus, which may reflect the proliferative nature of the smooth muscle in these lesions. Given the cytoplasmic staining in leiomyomas and aberrant nuclear expression in leiomyosarcoma, smoothelin expression, in conjunction with the standard histological features, may help in distinguishing benign and malignant smooth muscle neoplasm of the uterus.

Case Report: Unilateral Ovarian Fibroma in a 22-year-old Woman with Gorlin Syndrome

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Nevoid basal-cell carcinoma (Gorlin) syndrome is an inherited disorder characterized by malignancies of the skin and other organs, skeletal abnormalities, and congenital malformations. The syndrome follows an autosomal dominant inheritance pattern and is associated with mutation of the PTCH tumour-suppressor gene located on chromosome 9q22.3. We present a case of a 22-year-old woman with unilateral ovarian fibroma, falx cerebri calcifications, and odontogenic keratocysts. The patient had not yet developed skin manifestations. The patient initially presented at age 7 with complications due to recurrent odontogenic keratocysts. At age 20 she developed irregular menses and mild hirsutism and was investigated with imaging studies revealing a 10 cm complex midline pelvic mass. Upon salpingo-oophyrectomy, the pathological findings revealed a calcified, cystically degenerative ovarian fibroma. The patient was subsequently diagnosed with Gorlin syndrome after fulfilling two major and one minor diagnostic criteria. Unique features of our case include manner of diagnosis, lack of skin manifestations, and unilaterality of the ovarian fibroma. We discuss the pathologic findings and immunohistochemical profile of the tumour and the unique mode of pathologic presentation of ovarian fibromas in this syndrome compared to sporadic cases. Diagnostic criteria of Gorlin syndrome will be presented. Our case highlights the importance of early diagnosis of such cases to help direct patient care to early detect and alleviate any future co-morbities.

Pathology of Second Trimester Pregnancy Failure Associated with Abnormal Maternal Serum Screening

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Background: Abnormal placentation is central to the principle pathologies of pregnancy. Maternal serum markers, combined with Doppler ultrasound evaluation, provide an opportunity to identify patients at risk for placentation related pregnancy complications and facilitates early specialist referral in the second trimester.

Objective: To describe the pathology of early pregnancy failure associated with abnormal maternal serum screening.

Methods: We retrospectively reviewed the placental pathology from 20 pregnancies with placentation related adverse second trimester outcome with comparison to gestational age matched controls. All women had abnormal second trimester serum inhibin or - human chorionic gonadotrophin. Pathologically, emphasis was placed on categorizing the type and distribution of syncytial knotting and on trophoblast degeneration patterns.

Results: Frequency of villous infarction, atherosis, increased perivillous fibrinoid, fetal vascular obstruction, laminar decidual necrosis, distal villous hypoplasia and a distinct but

underreported serrated/wave syncytial knot pattern were each significantly increased in cases versus controls (p values ≤.05). Novel observations included: (a) a process of villous degeneration resulting in what we term "pale avascular villi", (b) a spindle cell proliferation at the basal plate that we term a "basal plate plaque" and (c) a linear arrangement of nuclei in normal syncytiotrophoblast that likely represents a basis for the distinctive pathological serrated/wave knot pattern. Conclusion: Detailed categorization of syncytial knot type and trophoblast morphology is achievable in routine histological sections and provides valuable diagnostic information as well as insights into both normal and abnormal placental development.

Phyllodes Tumour with Malignant Morphology of the Vulva: A Case Report and Review of the Literature

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Phyllodes tumour arising from the mammary-like tissue of the vulva is rare, and such a tumour with malignant morphology has, to our knowledge, not been reported in the literature. We report a case of a 61-year-old nulligravida woman who presented with an exophytic mass protruding from the clitoral area. The wide local excision specimen contained a tumour with sarcomatous features similar to those of malignant phyllodes tumour of the breast. Whether the biologic behaviour of the vulvar tumour carries malignant potential remains to be established on long term follow-up.

Helicobacter Pylori Detection Using a Modified Giemsa Stain

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Objectives: Gastritis caused by *Helicobacter pylori* (HP) continues to be a worldwide health challenge. The gold standard for diagnosis of HP infection remains morphologic identification of the organisms in gastric biopsies. Over time numerous stains have been applied to visualize HP, with immunohistochemical (IHC) detection being the definitive diagnostic method. However, IHC continues to be far more expensive than "special stains." We describe a modified Giemsa stain (mGS) utilizing a pre-set kit for rapid detection of HP organisms.
Methods: Slides were stained for 30 seconds in 0.12% phosphate buffered thiazine solution (Harleco Hemacolor Solution #3), washed and counterstained for 30sec in 0.125% Phosphate Buffered Eosin Solution (Harleco Hemacolor Solution #2), washed, dehydrated in 100% EtOH and cleared in xylene. Twenty gastric antral biopsies, ten HP-positive and ten HP-negative cases, were stained with the mGS and compared to an alcian yellow-toluidine blue stain (AYT); IHC served as control. Time to detection of organism, staining intensity and visibility were recorded. The Spearman rank-order correlation coefficient (r) was used to assess bivariate association.
Results: Nine of ten cases were positive for HP by mGS and IHC, as previously documented by AYT. One case did not yield any further material for examination due to exhaustion of archival tissue. Mean detection time for organism by IHC was calculated at 5.12sec (range: 3.8–7.4), by mGS 6.24sec (range: 6.2–9.8) and by AYT 12.62 sec (range: 6.3–18.4). Detection by mGS correlated positively with the detection time by IHC ($r = 0.06$). Additionally, 44% ($n = 4$) of HP-positive cases were graded higher by mGS than in comparison to AYT.
Conclusion: While the described method has only been applied to a small sample size, the data suggests that the mGS is a useful and cost-effective alternative to IHC in the majority of HP-infected cases for use as a rapid screening tool.

Chondroid Syringoma with Extensive Ossification: A Case Report and Review of the Literature

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Chondroid syringoma, also known as mixed tumour of the skin, is an uncommon benign tumour of the sweat gland, resembling a pleomorphic adenoma of the salivary gland. Ossification in this tumour is extremely rare and has been reported in only a few cases. Here we report a case of a 63-year-old man who presented with a slow growing tumour on his forehead. Clinically, the lesion was 2.2 cm in diameter, freely mobile, and excised completely. Histological examination of the biopsy specimen reveals a well-circumscribed tumour, involving the mid and deep dermis. Tumour cells form clusters, solid cores and ducts, embedded in a fibrous and myxoid/chondroid stroma. The ducts are lined by two layers of cuboidal cells with occasional eosinophilic material in the lumen. There is extensive ossification with marrow formation. The morphological features are diagnostic for chondroid syringoma with extensive ossification. The presence of extensive ossification with marrow formation in chondroid syringoma is a rare histologic feature and its awareness helps prevent diagnostic errors.

What Are Those Clear Cells?

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Background: Clear cells that are encountered on endometrial biopsies (EB) with cancer commonly represent secretory changes or clear cell changes within areas of squamous metaplasia in the context of endometrioid adenocarcinoma. However, their presence may also indicate a clear cell carcinoma (CCC) component.

We hypothesize that clear cells occurring in the setting of squamous metaplasia expresses P63 or cytokeratin 5/6 (CK5/6) , those occurring in the setting of secretory like changes expresses estrogen receptors (ER) and those representing true clear cell carcinoma demonstrated high KI67 index. The aim of this study was to examine the role of immunohistochemistry as an adjunct diagnostic tool.

Design: 116 consecutive in-house cases of EB with cancer were reviewed and 38 cases with endometrial adenocarcinoma containing areas of clear cells were identified. The immunohistochemical profile was studied using a panel of antibodies against P63, CK5/6, ER and Ki67. Immunostaining within the clear cell area was considered positive when > 10% of the cells were positive. High KI67 index was defined in cases with ≥50% positive nuclei. Fisher exact test was used to examine differences between the groups. Likelihood ratio (LR) > 1 indicates an increased probability that the target is present, and an LR < 1 indicates a decreased probability that the target is present.

Results: Routine histopathologic evaluation revealed 28/38 cases of endometrioid and 10 cases of nonendometrioid cell type (5 CCC, 4 mixed and 1 serous). The clear cell area was positive for P63 in 12/38 (31.6%), CK 5/6 in 18/38 (39.5%), ER in 25/38 (65.8%) and high Ki67 index in 11/38 (28.9%). Profile#1: [P63 positive or CK5/6 positive or ER positive and Ki67-low] was significantly associated with endometrioid type of adenocarcinoma (78.6% in endometrioid vs. 10% nonendometrioid; $p<0.001$). Profile #2: [P63 negative, CK 5/6 negative, ER negative and Ki67-high] was significantly associated with nonendometrioid type (0% in endometrioid vs. 40% nonendometrioid; $p=0.003$). The positive LR was 7.8 and negative LR was 0.2 for Profile#1 in identifying clear cells in the context of endometrioid histology. The positive LR was 0 and negative LR was 0.238 for Profile#2 in identifying clear cells in the context of endometrioid histology.

Conclusion: Immunohistochemistry may be used as an adjunct tool in the interpretation of clear cells that are encountered on EB. The impact of identifying a clear cell component has substantial clinical significance particular in view of the recent suggestion to limit complete surgical staging to high risk patients.

Fatal Cerebral Microangiopathy Following H1N1 Infection

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Introduction: The influenza A (H1N1) 2009 pandemic resulted in a significant number of deaths in Canada, (including 3 in Nova Scotia) and significant morbidity, largely from respiratory complications, including coagulopathy from external membrane oxygenation

(ECMO). Non-fatal encephalopathy in H1N1 positive patients has been reported, but histological findings have not been documented.

Case History: An otherwise healthy 43-year-old man developed acute confusion; a H1N1 swab was positive. He did not have respiratory symptoms but required respiratory support when he became severely neurologically compromised. He died 8 days after onset.

Results: The brain showed petechial hemorrhage of the inferior temporal cortex and amygdala bilaterally, with minmal encroachment of white matter; there was also recent hemorrhage in the neurohypophysis. Microscopically there was absent or degenerating endothelial cells within tubular ghosts of capillaries. There was negligible perivascular inflammation and no microthrombus.

Discussion: We suspect that a parainfectious, possibly cytokine mediated attack on selected brain capillaries, triggered apoptosis of endothelial cells. The classic patterns of para- or post-infectious encephalitis, acute disseminated encephalomyelitis (ADEM) and acute hemorrhagic leukoencephalopathy (AHLE) were absent. A literature search revealed no similar case report, and an informal survey of Canadian and some American neuropathologists also failed to identify a similar case.

Conclusion: The observed findings may reflect an idiosyncratic reaction to H1N1 by brain capillaries; whether cerebral microangiopathy underlies non-fatal cases of H1N1-associated encephalopathy remains undetermined.

Sudden Death in Infants with Congenital Heart Disease

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Objective: Despite advances in modern diagnostics and therapy, sudden death remains a risk for infants with congenital heart disease (CHD). Medical examiners need to be aware that CHD is a diagnostic consideration in cases of sudden unexpected infant death (SUID), and be aware of the spectrum of CHD that may cause SUID.

Methods: This retrospective descriptive study is based upon the 1991-2007 case files of the Miami-Dade County ME Department. Infants included were those with a cause of death attributed to CHD or its complications. Data recorded included: age, sex, race, cause and manner of death, circumstances of death, relevant clinical history, type of examination (autopsy/external examination), and autopsy findings. Basic statistics were used to analyze the data.

Results: The database included 53,500 cases, 799 of which were infants. Twenty-eight infants (3.5%) were certified as CHD-related deaths. Twenty-two cases (79%) underwent autopsy; of those, 2 had obstructive lesions (bicuspid aortic stenosis, cor triatriatum), 8 had flow lesions (VSD, ASD, endocardial cushion defects, tetralogy of Fallot, total anomalous pulmonary venous return), 6 had 'other' lesions (hypoplastic left heart syndrome, double outlet right ventricle, truncus arteriosus), 2 had mixed lesions (combination of obstructive and flow lesions), 2 had coronary artery anomalies, and 2 cases were excluded from further analysis due to insufficient data. Seven infants had an antemortem diagnosis of CHD, of which 4 had prior cardiac surgery; 7 infants had a history of non-specific symptoms, and 5 infants died within two weeks of birth.

Conclusion: Although uncommon, CHD remains an important diagnostic consideration for SUID. Forensic pathologists need to be familiar with the wide spectrum and morphology of cardiac malformations that may present in this way, and be aware that even complex cardiac defects may not be diagnosed or even symptomatic prior to sudden infant death.

When Infants Die Suddenly, is Routine Screening for Metabolic Diseases Warranted?

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Objective: It is very rare for inborn errors of metabolism (IEM) to cause sudden, unexpected death of infants. Yet, post-mortem metabolic screening is often reflexively ordered during an infant death investigation, even in the

absence of macroscopic autopsy findings to suggest IEM. This retrospective descriptive study examines the impact of routine metabolic screening of infants in a medical examiner's population.

Methods: Miami-Dade County sudden death investigation files (1999-2007 inclusive) were reviewed. Inclusion criteria included: live born infants ≤12 months, an autopsy must have been performed, and the case file must have been complete. Data recorded included pertinent demographic information, circumstances of death and other relevant histories, and the results of all components of the autopsy. Basic statistics were used to analyze the data.

Results: 251 cases met inclusion criteria. One case was certified as death due to IEM, and one case was certified as death due to probable IEM. Nine cases had screens with a slightly abnormal metabolic profile (seven of which were mildly elevated CAH-17 OHP values), but the metabolic derangements were not determined to be the cause of death. One case had autopsy findings highly suggestive of IEM, but screening tests were negative.

Conclusion: Although rare, IEM may cause the sudden and unexpected death of infants, and can be suggested from findings at autopsy. Routine utilization of screening tests in cases without historical or autopsy features suggestive of IEM did not detect unsuspected cases. Within the appropriate investigative and autopsy contexts, judicious use of metabolic screening tests is warranted.

Basal Cell Carcinoma with Pilomatrical Differentiation: A Case Report and Review of the Literature

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Background: Basal cell carcinoma (BCC) of skin is the most common malignant neoplasm of humans. Histologically BCCs are classified into undifferentiated (solid) and differentiated types. A variety of specific cell lineage differentiation can be observed within differentiated BCCs such as keratotic, infundibulocystic, follicular, eccrine, pilomatrical, and pleomophic BCCs. Although these differentiation patterns do not impact prognosis, they can produce diagnostic difficulties in routine pathology practice. We present a case of BCC with pilomatrical differentiation, an extremely rare variant, with only 14 cases described in the literature.

Case Report: A 66-year-old male patient underwent excision of a 1.0 cm tan skin nodule from left hand. Histologically this well circumscribed dermal tumour showed areas of classic BCC and sheets of eosinophilic shadow cells. A diagnosis of BCC with pilomatrical differentiation was made. The differential diagnosis of pilomatrical carcinoma was ruled out by the absence of cytologic atypia, necrosis, high mitoses and infiltrative growth pattern.

Conclusion: We believe that recognition of this distinct, extremely rare, subtype of BCC is important in routine pathology practice to avoid confusion with other malignant tumours for which prognosis and follow up are different.

Collision Tumour: Primary Lung Adenocarcinoma Colliding with Metastatic Papillary Thyroid Carcinoma

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Collision tumours are rare entities composed of two histologically distinct neoplasms coinciding in adjacent anatomic proximity. The 2 malignancies may originate from the same organ or occur as metastases from other sites. Here, we report a collision tumour of metastatic papillary thyroid carcinoma and primary lung adenocarcimoma occurring in the lung of a 60-year-old-male. The patient has a history of papillary thyroid carcinoma, status post total thyroidectomy with neck dissection and post operative radiation. He presented with multiple non-calcified pulmonary nodules. The largest nodule is 4 cm in size, located in the left lower lobe. The biopsy of this nodule showed highly atypical glandular cells, consistent with adenocarcinoma. A lobectomy of left lower lobe with lymph node dissection was performed. Histological examination of the lobectomy specimen showed moderately differentiated adenocarcinoma and foci of metastatic papillary thyroid carcinoma with multiple psammoma bodies. High power view revealed the presence of nuclear

grooves in tumour cells of metastatic papillary thyroid carcinoma but not in lung adenocarcinoma. Both cancers were infiltrative in lung tissue where the histologically distinct tumour types approximated one another but were not inter-mixed. Immunohistochemical stain of thyroglobulin showed positive staining in cells of metastatic papillary thyroid carcinoma but not in lung adenocarcinoma, supporting the diagnosis of collision tumour. Despite the rarity of collision tumours, accurate identification and recognition of such tumours are important as treatment plan and prognosis are dependent on the individual biological aggressiveness of each of the tumour components.

Morphological and Immunohistochemical Features of Angiomyofibroblastoma: A Case Report with Review of the Literature

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Angiomyofibroblastoma is a rare tumour seen predominantly in women of childbearing age. Here we report a case of angiomyofibroblastoma with unusual immunohistochemical features in a 62-year-old woman. The patient presented with a 9.5 cm mass in her left labium for one year. Microscopic examination of surgical resection specimen showed a well-circumscribed mass with alternating paucicellular and hypercellular areas in the background of an edematous and collagenous stroma with vascular proliferation. Tumour cells showed high nuclear/cytoplasmic ratio, oval nuclei with finely granular chromatin, inconspicuous nucleoli and a few mitoses (3 per 10 High Power Fields). Immunohistochemically, these cells were positive for vimentin, estrogen receptors and progesterone receptors, and negative for S-100, HMB45, BerEp4, cytokeratins, synaptophysin, CD34 and E-cadherin. The above immunohistochemical findings helped us to rule out melanoma, adenocarcinoma, neuroendocrine tumours and metastases from the breast origin. Considering the overall morphological features of the tumour, it was diagnostic of angiomyofibroblastoma. Although a literature review shows that angiomyofibroblastomas are generally diffusely positive for desmin and focally positive for -smooth muscle actin (SMA) with only very rare exceptions, desmin and SMA immunostains were negative in our case. We like to emphasize that it is important to be aware of the potential variations in the immunophenotypes of angiomyofibroblastomas in order to make a correct diagnosis.

Strong Correlation between HO-1 Expression and PTEN Deletion in Prostate Cancer and Its Prognostic Value

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Introduction: Over-expression of heme oxygenase-1 (HO1), a sensor and regulator of oxidative stress and tissue redox homeostasis, and PTEN deletions, a well-known tumour suppressor gene, has been reported in prostate cancer (PCA). Herein, we assessed HO1 expression and PTEN deletions, and their prognostic values, in a large cohort of men with localized PCA.

Methods and Results: HO1 expression was scored semi-quantitatively and PTEN status was assessed by a four-color interphase FISH performed on an outcome TMA of 196 men with localized PCA. There was statistically significant difference in epithelial HO1expression between different path diagnoses (BN: 1.49 + 1.03; HGPIN: 1.07 + 0.87; PCA: 1.20 + 0.95) ($p = .03$). Also a weak correlation between both epithelial and stromal HO1 expression and Gleason score was detected, though in opposite direction. More importantly, there was strong positive correlation between epithelial HO1 expression and PTEN deletions, $p < .0001$. Although neither showed significant association with PSA relapse, the combined status of both correlated with disease progression (Log-rank test, $p = 0.01$). Inhibition of HO1 by siRNA in LNCaP and PC3 cells (PTEN-null cells) where PTEN is restored significantly enhanced cell's susceptibility to apoptosis *in-vitro* and inhibited tumour growth in mice compared to control cells where PTEN is not rescued.

Discussion: We provide novel evidence for strong

correlation between epithelial HO1 expression and PTEN deletions in relation to patient's outcome. This cooperation is supported by data using PCA cell lines where both HO1 and PTEN were manipulated. These findings will contribute to understand the underlying genetic pathways leading to disease progression and could potentially lead to discovery of novel therapeutic modalities for advanced PCA.

Water-Clear Cell Adenoma of Parathyroid, A Case Report and Literature Review

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Parathyroid adenoma of water-clear cell type is an extremely rare neoplasm, and only very few cases have been reported up to date. Here we report another case of water-clear cell adenoma of the parathyroid in a 60-year old female patient who presented with hypercalcemia and high PTH level. Sestamibi scan showed a possible parathyroid adenoma involving the right superior parathyroid gland. The surgically excised specimen had a weight of 13.3 grams and contained a cystic hemorrhagic mass measuring $1 \times 1 \times 0.8$ cm. Histologically, the tumour mass was surrounded by a thin fibrous capsule with a compressed thin rim of normal parathyroid tissue. High power examination revealed the tumour to be composed of cells with a vacuolated cytoplasm, with minimal nuclear pleomorphism. Immunohistochemical analysis demonstrated positive staining for PTH and chromogranin. Histological examination of the right inferior parathyroid gland showed unremarkable parathyroid tissue. Post-operatively, the patient's serum calcium level returned to normal. All these findings support our diagnosis of water-clear cell adenoma of the parathyroid gland. The patient's parathyroid function is being followed. In this report, we also reviewed related literature and compared our case with similar cases reported before.

Iatrogenic Blood Loss at Capital District Health Authority: An Institution-Wide Retrospective Review of Phlebotomy Practice

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Background: Blood conservation strategies have been proposed as a solution for both patient safety and utilization issues of the blood supply. A key component of these strategies is the reduction of blood loss due to laboratory testing. This issue of quantifying iatrogenic blood loss has been examined in various specific patient care settings, such as intensive care wards; however, there is no literature quantifying iatrogenic blood loss in day-to-day patient care in Canadian hospitals. We present a novel informatics-driven approach to quantify district-wide phlebotomy volumes. This data will allow our institution to target resources to reduce unnecessary iatrogenic blood loss and improve patient care.

Methods: A retrospective review of all blood tests performed on inpatients in Capital District Health Authority (CDHA) facilities over a ten-week period (Nov. 2008- Jan. 2009) was done using data from the Laboratory Information System (LIS). The final data set consisted of 213,377 records of individual tests, matched to data on patient demographics, site of care, and care team. Phlebotomy volumes were estimated based on the least amount of blood required by CDHA laboratory guidelines for a given set of tests. Results: Records on 3,804 inpatients in 12 different facilities were included in the analyses (1,796 females, 2008 males, mean age = 63.4 years). The median number of orders per patient was 5 (range 1–160), while the median number of individual tests was 24 (range 1-1207). Median estimated blood loss to phlebotomy was 40.7 mL (range 1.7 – 1243.6 mL).

Conclusions: It appears that most inpatients in CDHA facilities do not have significant iatrogenic blood losses. However, given the broad ranges observed, there is a heavily tested subpopulation that may benefit from intervention in clinical and/or laboratory practice.

Primary Soft Tissue (Extraosseous) Aneurysmal Bone Cyst: Report of a Case with Myositis Ossificans-Like Features and USP6 Gene Rearrangement As Detected By Fluorescence In Situ Hybridization

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Primary aneurysmal bone cyst (ABC) is a rare, benign lesion characterized by recurrent USP6 gene locus rearrangement in the majority of cases. Recent identification of the same USP6 rearrangement in lesions histologically and radiographically compatible with the benign soft tissue lesion myositis ossificans (MO), indicate a subset of "classic" MO cases are classifiable as primary soft-tissue ABC with USP6 rearrangements. Nevertheless, soft tissue lesions demonstrating co-existent histologic features of ABC and MO are exceedingly rare.

We report the case of a soft tissue lesion in the right arm of a 17-year-old woman that demonstrated histologic features of MO on initial biopsy but eventually exhibited histologic features of both ABC and MO on subsequent biopsy. Fluorescence in situ hybridization (FISH) identified the USP6 gene rearrangement characteristic of primary ABC in the lesion. To our knowledge, this is the first reported case in the English-language literature of a histologically well-developed primary soft tissue ABC with MO-like features confirmed to harbour the USP6 gene rearrangement by FISH. The presence of the USP6 gene rearrangement in a primary soft tissue ABC that appeared to evolve from an earlier MO-like lesion lends support to the concept that a subset of soft tissue lesions with histologic features of MO may represent the early phase of soft tissue ABC.

Role of Immunohistochemistry (IHC) in the Diagnosis of Lung Neoplasms – What Is Important Histology or IHC?

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Background: The diagnosis of lung neoplasms is subject to interobserver variability problems, particularly in diagnosis of poorly differentiated tumours. IHC may help define tumour cell type. However, its role is currently uncertain as most antibodies do not provide 100% specificity for cell type.

Design: One hundred and twelve lung neoplasms including 72 transbronchial biopsies and 40 wedge biopsies were studied with IHC for CK5/6; p63; TTF-1; Napsin A; Synaptophysin and Chromogranin. These cases included 35 large cell carcinoma (LCC), 34 squamous cell carcinoma (SCC), 23 adenocarcinoma (AC), 8 adenosquamous carcinomas (ASQ), 7 large cell neuroendocrine carcinomas (LCNEC), and 5 small cell carcinomas (SCLC). Six arbitrary diagnostic categories (I-diagnoses) were defined using the tumour immunoprofile as follows: I-SCC (CK5/6 or p63 +), I-AC (TTF-1 or Napsin A +), I-SCLC (cell size + Synaptophysin or chromogranin positive), I-LCNEC (cell size + synaptophysin or chromogranin positive), I-ASQ (CK5/6 or p63 and TTF-1, or Napsin A positive), I-LCC (no marker +). Diagnoses rendered by standard WHO criteria using histopathology and hematoxylin and eosin stained slides were compared with I-diagnoses using kappa statistics, by cell type.

Result: Kappa values for paired comparisons were 0.54, 0.5, 0.4, 0.1, and 0.1 for SCC-"I-SCC," LCNEC-"I-LCNEC," AC-"I-AC," LCC-"I-LCC," and ASQ-"I-ASQ" respectively. Kappa value for SCLC-"I-SCLC" was 1.0.

Conclusion: There is poor to moderate agreement between diagnoses of SCC, AC, LCC, ASQ and LCNEC in contrast, there is perfect concordance between the diagnosis of SCC and its "I-diagnosis" suggesting that should include immunoprofile in its classification scheme.

Value of Ki67 In Locally Advanced Breast Cancer Treated With Neoadjuvant Chemotherapy

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Background: Tumour expression of the proliferation antigen Ki67 is used to assess the prognosis of cancer patients. This study was designed to investigate the impact of neoadjuvant therapy (NAT) on Ki67 expression and its

relationship with chemotherapeutic response in locally advanced breast cancer (LABC).

Method: Patients with LABC (stage 2B or stage 3) who were entered into the prospective LABC database between 2002-2008 were included. The neoadjuvant regimen in these patients included anthracycline based chemotherapy (doxorubicin or epirubicin) and in patients with ER/PR negative tumours taxanes (paclitaxel, docetaxel). Ki67 was evaluated in cases with available tissue on preNAT core biopsy and postNAT specimens. Consecutive sections were studied by immunohistochemistry using antibodies directed against ER, PR, HER2/neu and Ki67 (SP6 1:800 Labvision). The percentage of Ki67 positive neoplastic cells (index) was determined using Ventana Image Analysis System (Tripath Imaging Inc, Tucson Ariz USA). Association between Ki67 pre and postNAT and clinical response and biomarkers was evaluated.

Results: 149 patients were enrolled in the LABC database. 77 had tissue available for immunohistochemistry (76 cases had preNAT cores, 63 postNAT and 62 had both pre and postNAT specimens).

High Ki67 index on preNAT biopsy correlated with Nottingham grade III (p = 0.03), ER- (p = .0002), PR- (p = .003) but not Her2/neu+ (p = 0.5), nodal status (p = .19) or size (p = .51). Ki67 index on preNAT biopsy was not a significant predictor of clinical response (p = .57).

High Ki67 index on postNAT specimen was associated with clinical response (p = .03).

Change in Ki67 index between preNAT and postNAT specimens occurred in 61/62 cases (38 decreased, 23 increased). Decreased Ki67 index was associated with ER+ status (p = .0059) and increased Ki67 index was associated with triple negative (p = .037). Decreased Ki67 index was associated with clinical response (p = .0047).

Conclusion: There is a significant association between high preNAT Ki67 index and hormone receptor negativity and high tumour grade. ER+ cases are more likely to show decrease Ki67 following NAT while triple negative cases are more likely to show increase in Ki67 following NAT.

This study indicates that in LABC patients Ki67 index in postNAT specimen is a marker of response to NAT. Furthermore, decreased Ki67 index between pre and postNAT correlates with clinical response.

miR-375 is Markedly Overexpressed in Merkel Cell Carcinoma

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Background: MicroRNAs (miRNAs) are small, non-coding, regulatory RNAs encoded in the genome of animals, plants, and insects. Dysregulation of miRNA expression has been observed in many diseases, including cancer. We speculated that miRNAs are dysregulated in Merkel cell carcinoma (MCC), and that specific miRNAs are involved in MCC pathogenesis.

Design: To address this, we began by comparing the miRNA expression profiles of MCC tumours and normal skin using an Agilent microarray. Archival formalin fixed, paraffin embedded (FFPE) tissue was used for this analysis. We have previously confirmed the validity of using FFPE tissue to profile miRNAs using an Agilent array platform (J Mol Diagn 2008;10(6):513-9). Data validation was carried out first with quantitative real time RT-PCR analysis and subsequently with a novel cloning and sequencing profiling method.

Results: Unsupervised hierarchical clustering clearly illustrated significant differences in miRNA expression profiles between MCC and normal skin. Supervised analysis identified 5 up-regulated and 10 down-regulated miRNAs in MCC relative to normal controls. Validation confirmed both aberrantly overexpressed and underexpressed miRNAs in MCC. Certain miRNAs appear to be of great interest in MCC: miR-375 expression was 310 fold increased, while others miRNAs were up to 500 fold decreased, as compared to normal skin.

Conclusion: Our studies suggest that miRNAs are dysregulated in Merkel Cell Carcinoma and that miR-375 in particular may play a role in its initiation and progression.

Pseudoglandular Pattern in a Myxoid Adrenocortical Adenoma

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Myxoid change in adrenocortical neoplasms is very rare and was initially identified in 1979. Since then only 27 cases of myxoid change in adrenocortical neoplasms have been described. Of these, only 10 have demonstrated a pseudoglandular pattern. We describe a case of myxoid adrenocortical adenoma with pseudoglandular pattern occurring in a 63-year-old woman. Clinical history and imaging characteristics were compatible with an adrenocortical adenoma and a left adrenalectomy was performed. Grossly, a yellow, well-circumscribed nodule was found measuring 2.5 × 2.5 × 1.5 cm. By light microscopy, the tumour was composed of islands and fascicles of cells with abundant vacuolated cytoplasm and a smaller component of eosinophilic cells. Areas of myxoid change with a pseudoglandular pattern were seen. No significant atypia, mitoses, or vascular invasion were present. Immunohistochemical staining revealed the tumour cells to be strongly positive for Mart-1 and vimentin, weakly positive for inhibin and synaptophysin, and negative for monokeratin and chromogranin A. The material within the pseudoglandular spaces stained positive for alcian blue and negative for PAS with diastase. Myxoid change has been described in both adrenocortical adenomas and carcinomas. In cases where the myxoid change is extensive, the differential diagnosis of well-differentiated metastatic adenocarcinoma should be considered and excluded using cytokeratin immunostains. The pathogenesis of this variant of adrenocortical tumour is not understood and the primary importance of recognizing prominent myxoid change is to avoid mistaking it for other myxoid lesions, including metastatic adenocarcinoma. The identification of the typical Mart-1, synaptophysin, and alpha inhibin positive immunophenotype of a conventional adrenocortical adenoma may help to lead to the correct diagnosis.

Comparison of LHSC and Bethesda Thyroid FNA Diagnostic Categories

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Purpose: In 2003 London Health Sciences Centre (LHSC) cytology adopted a new reporting system for thyroid fine needle aspiration (FNA). Recently, the Bethesda system for standard reporting was published. We compared LHSC and Bethesda reporting systems and malignancy rates.
Materials and Methods: Follow-up excision results of thyroid FNAs at LHSC (Mar 2003-9) were obtained. Malignancy rates were calculated and compared to Bethesda.
Results: For 5,274 FNAs, 20% were unsatisfactory; 80% were adequate with 1,585 resections.

LHSC	Malignancy	Bethesda	Malignancy
Unsatisfactory	9%	Unsatisfactory/Nondiagnostic (and cyst fluid)	1–4%
Benign	3%	Benign	0–3%
Follicular lesion	21%	Atypia/follicular lesion undetermined significance	5–15%
Follicular neoplasm	14%	Follicular neoplasm / suspicious for neoplasm	15–30%
Hurthle cell lesion	12%	Follicular neoplasm/ suspicious for neoplasm	15–30%
Suspicious for Pap ca	80%	Suspicious for malignancy	60–75%
Positive for Pap ca	98%	Malignant	97–99%
Cystic contents	13%	Unsatisfactory/Nondiagnostic	1–4%
Indeterminate for Pap ca	50%	Atypia/follicular lesion undetermined significance	5–15%

Conclusions: LHSC and Bethesda diagnostic categories were similar with variation in the atypia/ follicular lesion group. Malignancy rates in most LHSC categories were validated with Bethesda.

Quantitative Imaging Cytometry: Evaluation of Unstained Tissue Morphology Obtained by Laser Light Absorption

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Objectives: Digitized slides are increasingly used for diagnostic purposes, but scanned slides containing both conventional morphology and multiparametric protein expression data are still developmental. Multiplexed

immunohistochemical stains are technically challenging, and chromogenic stains interfere with fluorescent stains via unwanted light absorption. One solution is to visualize morphology on unstained tissue. We evaluated a modality of quantitative imaging cytometry that produces images using laser light absorption data, in order to determine its suitability for morphologic diagnosis and localization of staining to particular cell/tissue compartments.

Methods: De-paraffinized, hydrated sections of a variety of tissues (n = 20) and cytology or bone marrow samples (n = 30) placed in a 96 well plate were scanned on an iCys imaging cytometer (CompuCyte) which produces raster images of specimen fields by combining stage movement in the x-axis and mirror-galvanometer-controlled movement of laser(s) in the y-axis. A 40× objective, producing a pixel size of 0.25 μm^2, was used in most cases. Laser light absorption signal reaching an offset photodiode was manipulated by instrument software to produce "shaded relief" images analogous to differential interference contrast microscopy.

Results: Tissue architecture and patterns of cell dispersion (in cytology samples) were easily identified, as were N/C ratio, character of cytoplasm, nuclear chromatin texture, presence of nucleoli, and character of extracellular material, including infectious organisms. Most cell types could be identified.

Conclusion: Laser light absorption "shaded relief" morphology was consistently informative in examination of unstained tissue sections and cytologic preparations. This data can be captured simultaneously with multichannel fluorescence, thus providing a useful morphology component for high-content virtual slides in both histology and cytology.

Glomus Tumour of the Jejunum: A Previously Unreported Cause of Gastrointestinal Hemorrhage

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Gastrointestinal (GI) glomus tumours are rare entities and are only exceptionally identified in the small bowel. We report the first case of a symptomatic glomus tumour of the jejunal mucosa in an 18-year-old male who presented with GI haemorrhage. The patient's admission haemoglobin of 65 g/L subsequently plunged to 49 g/L prompting multiple transfusions and investigations for active GI bleeding. An enhancing small bowel mucosal lesion was detected by CT angiography. Resection of a segment of jejunum identified an ulcerated, 0.7 cm mucosal mass. Histologically the tumour consisted of an epithelioid neoplasm with tumour cells arranged in nests and cords, focally surrounding large blood vessels. No features of malignancy were identified. Immunohistochemical investigations excluded the more common epithelial and mesenchymal diagnostic considerations and allowed diagnosis of a gastrointestinal glomus tumour. The patient had two previously resected soft tissue glomangiomas, raising the possibility of a genetic predisposition to glomus spectrum tumours. Non-gastrointestinal stromal tumour (GIST) mesenchymal neoplasms of the small bowel are rare and of these glomus tumour is exceptional. Although prior reported cases suggest that small bowel glomus tumours are preferentially subserosal and of little clinical significance, this case illustrates that mucosal-based glomus tumours of the small bowel can produce severe clinically significant GI haemorrhage.

Cardiovascular Manifestations of Fabry Disease

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Aim: To study the clinical, histopathological, electron microscopic findings of Fabry disease, involving the heart.

Materials and Methods: The case records from 1995–2010 at UHN hospital, Toronto and HHS, McMaster University were reviewed for Fabry disease involving the heart. The clinical data including patient's age, sex, presenting symptoms and the procedure they underwent were reviewed. The histological pathological findings and the em findings were reviewed.

Results: There were seven cases of Fabry disease (5 UHN, 2 HHS), five male and two female patients. Age at surgery

ranged from 27 years to 59 years. A family history of Fabry's disease was present in 3 patients. Patients presented with symptoms of hypertrophic obstructive cardiomyopathy (3), congestive heart failure (2). On histological examination myocyte hypertrophy was seen in five cases, vacuolation of the myocytes in six, interstitial fibrosis in four, myocyte disarray seen in two and endocardial fibrosis in three cases. Vacuolation was also seen in the atrial appendage, internal mammary artery and mitral valve. In six of the seven cases electron microscopy was performed. All the cases showed intracellular lamellar structures consistent with Fabry disease.

Conclusion: Patients with Fabry disease of the heart most often present with symptoms of hypertrophic obstructive cardiomyopathy and a clinical diagnosis of hypertrophic cardiomyopathy is made. When myectomy is performed to relieve the symptoms, it can lead to a correct diagnosis. In those undergoing septal ablation, this opportunity is lost. Histopathological examination suggests and electron microscopic findings help confirm a diagnosis of Fabry disease. This is clinically very relevant since enzyme replacement therapy is available for patients with Fabry disease while the only option available for Hypertrophic cardiomyopathy is cardiac transplant. (All cases of clinical HCM should have an endomyocardial biopsy, to exclude Fabry disease and to confirm HCM.)

Intra-Operative Assessment of Breast Sentinal Lymph Node By Cytology Touch Preparation

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Background: The presence of metastatic disease in axillary lymph nodes is an important prognostic indicator of survival in breast cancer. Intra-operative touch imprint cytology (TIC) of sentinel lymph nodes (SLNs) avoids unnecessary axillary lymph node clearance. TIC is quick to perform, requires minimal tissue preparation, can be done using simple equipment and will provide acceptable details for interpretation. We investigated the accuracy of performing intra-operative assessment of SLN using TIC at our centre, and attempted to identify factors associated with false negative results.

Methods: The surgical reports of 187 women with operable breast cancer who underwent SLN biopsy during 2008/ 2009 were retrieved from the LIS, and reviewed. Results of intra-operative TIC of SLNs were compared with results of histological examination.

Results: A total of 42/187 women had SLN metastases on histological examination of which 15 were correctly detected by TIC. In only 3 cases were a positive TIC result not confirmed by histological examination. The sensitivity, specificity, negative predictive value and positive predictive value were 36%, 98%, 84% and 83% respectively. True positive results were significantly more likely to be associated with lympho-vascular invasion (p=.041), and false negative results were significantly more likely to be associated with lower grade (p=.049).

Conclusion: Intra-operative TIC on SLNs in women undergoing surgery for breast cancer has a high specificity and spares a significant number of women unnecessary axillary lymph node clearance. Sensitivity is low, resulting in additional surgery in a small proportion of patients, particularly associated with lower grade cancer

Identification and Characteristics of T-Cell Clonality among Patients with Incidental Lymphocytosis

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Identifying clonal rearrangements of the T cell receptor (TCR) is useful in the diagnosis of T cell leukemia. The independent significance of TCR clonality, however, is currently unclear. In this study, we evaluated 27 patients with clonal T cell lymphocytosis. All patients presented with peripheral lymphocytosis. Laboratory data including CBC, flow cytometry, PCR assay for TCR beta and gamma genes, blood film review and clinical findings were analyzed. The patients displayed a variety of co-morbidities; 7/27 (26%) patients had a co-existing hematologic malignancy, 3/27 (11%) had organomegaly, and none of the patients had clinical or serologic findings suggestive of autoimmune disease. None of the patients were anaemic and only one had

thrombocytopenia and neutropenia. The average duration of lymphocytosis was 21 months. Patients had an average a lymphocyte count of 4.97x109/L with an average of 75% being CD3+. PCR showed 11/27 (41%) had a clonal pattern of TCR Gamma, 2/27 (7%) had a clonal beta pattern and 14/27 (52%) demonstrated clonal rearrangements of both Gamma and Beta genes. Blood film review showed that the predominant morphology of atypical lymphocytes in only 36% patients, while small lymphocytes appeared as the predominant cell type in 64% patients. This study demonstrated heterogeneous clinical, morphological and phenotypic characteristics of patients with T cell receptor clonality. The significant portion of patients in this study who presented with T cell clonality in the absence of any other hematologic abnormality represents a T cell clonality of undetermined significance.

Arginase and YKL-40 are Novel Biomarkers in MDs and CMML

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Objectives: Based on murine models, we hypothesized that human MDS/CMML cells may over-express markers of, and/or be associated with expanded, immunosuppressive myeloid populations.
Background: SHIP-knockout mice are a model of MPN/MDS and contain alternatively (M2) activated macrophages, characterized by arginase 1 (Arg1)-mediated redirection of L-arginine metabolism away from cytotoxic NO and towards the production of healing intermediates, as well as over-expression and crystallization of the Th2-skewing chitinase-like protein Ym1.
Methods: Literature reviews/data-mining for features of M2-skewing in MDS/CMML. Enzymatic assay and/or Western blot to assess Arg1 and YKL-40 in MDS/CMML BM-MNCs.
Results: ARG1 is commonly up-regulated in MDS neutrophils, and the chitinase-like gene, CHI3L1 (encoding YKL-40, a human homologue of murine-Ym1), as well as the SHIP gene (INPP5D) are microarray-based MDS classifier. Moreover, a majority of CMML patients demonstrate high co-expression of ARG1 and CHI3L1 mRNA. Accordingly, mean arginase activity/expression and YKL-40 levels were significantly higher in MDS and CMML BM-MNCs, as compared to controls, and strikingly correlate with PB neutrophil counts.
Conclusions: Arginase and YKL-40, markers of suppressive immature myeloid cells and macrophages, are over-expressed in BM-MNCs of MDS/CMML patients, and may contribute to a pathological and immune-evasive BM micro-environment.

B-Cell Lymphoproliferative Disorders with Multiple Clonal Populations Demonstrated By Flow Cytometric Immunophenotyping

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Mature B lymphocytes exhibit allelic exclusion in that only a single class of Ig heavy chain and a single class of light chain , either kappa or lambda, are expressed. Mature B cell neoplasms often mimic normal B-cell differentiation. However, several reports indicate that B-cell malignant neoplasms may exhibit two or more clonal populations in the same patient. These may represent either separate clonal entities or provide evidence of clonal evolution or clonal divergence. We summarize our experience with three such cases.
Case 1: 83-year-old woman with absolute lymphocytosis ($5.14x10^9$/L). Two clonal B-cell populations co-expressing CD5 and CD23 demonstrated by immunophenotyping: one is lambda and the other kappa restricted.
Case 2: 85-year-old woman with eyelid lesion. Immunophenotyping demonstrated a kappa restricted B-cell population co-expressing CD5 and CD23, and a lambda restricted B-cell population expressing CD10.
Case 3: 73-year-old man with anemia and lymphocytosis. Immunophenotyping demonstrated three clonal B-cell

populations: i) CD5+CD20+CD23+kappa+(dim); ii) CD5+CD20+CD23+lambda+(dim) and iii) CD5-CD20+CD22+kappa+.Molecular diagnostic studies performed by PCR and GeneScan revealed clonal IgH gene rearrangements in all three cases.

Conclusion: Multiple clonal populations may occur in mature B cell lymphoproliferative disorders and may be demonstrated by flow cytometric immunophenotyping. Recognition of the presence of two or more light chain restricted B-cell populations is important in diagnosis and may have implications for residual disease monitoring. Molecular genetic analysis may add useful insight regarding the nature and origin of the clonal populations.

Additional Levels and Phosphohistone H3 (PPH3) Expression are Useful to Evaluate Proliferation Activity in Thin (T1x) Melanomas

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Objective: According to the 7th version of the AJCC Melanoma Staging, the presence of a single mitosis in a melanoma 1mm thick or less is sufficient to raise the stage from T1a to T1b. The objectives of this study were to evaluate whether additional levels of the lesions originally classified as T1a would ultimately change the staging, and to assess the utility of PPH3 immunostaining as an aid for detecting mitoses.

Methods: A total of 60 primary melanomas have been included. As a pilot study, we studied 30 cases of T1x melanomas distributed among 26 cases without mitosis (T1a) and 4 cases as controls with one mitosis/mm2 or more (T1b). The original H&E slides were reviewed and 6 levels were done every 50 μm. One level (at 100 μm) was immunostained with PPH3 mAb (1/100, Cell Signaling Technology, Inc., Boston, MA) with ALP-Fast red revelation. The number of stained nuclei, non-mitotic and mitotic, was counted per mm^2.

Data and Results Obtained: Additional levels revealed the presence of ≥1 mitosis/mm^2 in 9 (34%) melanomas initially reported as T1a. PPH3 revealed the presence of mitoses in 5 other cases confirmed as T1a based on the additional HEs. These cases were small-cell melanomas with basophilic nuclei. In total, 53% of the melanomas initially classified as T1a according to the 2010 AJCC classification were actually T1b.

Conclusion: The 7th AJCC melanoma staging system puts too much emphasis on the presence of a single mitosis that is highly subject to sampling effect. PPH3 immunostaining is useful as an additional tool to help identify mitoses.

Expression of the Stem-Cell Associated Protein Bmi-1 and Its Utility in Differentiating Nevocellular Nevi, Spitz Nevi and Melanoma: An Immunohistochemical Study

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Objective: BMI-1 is a gene involved in the maintenance of various stem cell lines, mainly through epigenetic silencing of other developmental regulatory genes. It has also been shown to play a role in cellular development and control of the cell cycle. BMI-1 overexpression has been linked to oncogenesis, and increased immunohistochemical expression has been demonstrated in some tumours. To date, the expression of BMI-1 has not been evaluated in melanocytic lesions. Here we evaluate whether there is any differential expression of BMI-1 in nevocellular nevi, Spitz nevi and melanoma.

Methods: Representative cases of nevocellular nevi (intradermal nevi) ($n = 2$), Spitz nevi ($n = 2$) and melanoma ($n = 2$) were selected from our slide archives. In addition, one case of Merkel cell carcinoma was selected as a positive control. Paraffin sections were cut and immunohistochemistry was performed on each using BMI-1 monoclonal antibody as per protocol. Interpretation was assessed as positive staining (at least moderate staining in >10% of target lesional cell nuclei) equivocal (weak staining or staining in <10% lesional cell nuclei), and negative (no staining in lesional cell nuclei).

Results: All six test cases showed diffuse positive staining in

lesional cells interpreted as positive. The control case also showed positive staining.

Conclusions: BMI-1 expression as assessed by immunohistochemical means does not offer discriminatory utility in the evaluation of melanocytic lesions as it appears to be an expressed protein in both benign melanocytic lesions (including Spitz nevi) as well as malignant melanoma.

The Mechanism of Neutrophil DNA NET Formation: An Ultrastructural Study

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Background: Neutrophil extracellular traps (NETs) are webs of DNA covered with antimicrobial molecules that constitute a newly described killing mechanism in innate immune defense (Brinkmann et. al, *Science* 2004;303). Neutrophils respond to *Staphylococcus aureus* and other bacteria via a novel process of NET formation.

Objectives: The objective of this observational study was to use live cell light microscopy and transmission electron microscopy to identify morphological changes of the nuclear envelope integrity during neutrophil activation and subsequent NET formation.

Methods: Neutrophils were purified and collected from humans, activated with the addition of *S. aureus* and then fixed at various time points. The images obtained from the electron microscope were correlated with images obtained from live cell imaging data.

Results: The formation of NETs is a highly complex process whereby the multi-lobular nucleus rapidly becomes rounded and condensed. The lumen of both the nuclear envelope and endoplasmic reticulum filled with nuclear chromatin strands. Numerous vesicles budded from the dilated nuclear envelope into the cytoplasm. The vesicles are filled with chromatin strands and eventually fuse with the plasma membrane. Eventually nuclear envelope breakdown occurred in a similar fashion to previously published studies (Salina et al, *Cell* 2002;108). DNA then filled the cytoplasm prior to being released extracellularly. Extracellular NETs form fibrillary structures made up of chromatin strands admixed with other proteins.

Conclusion: The neutrophil nucleus is a highly malleable structure with the capability to undergo drastic changes in nuclear morphology when activated by *S.aureus*. Neutrophils form DNA NETs from a highly complex process which includes nuclear envelope breakdown with the exocytosis of DNA containing vesicles (publication in review).

Cyst in Solitary Kidney

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A 17-year-old girl with a solitary kidney and a medical history of urinary tract infections during 7 years was referred to the renal clinic because of non nephrotic proteinuria, moderate arterial hypertension and microsopic hematuria. Renal function was normal. She was treated with angiotensin converting enzyme inhibitors. Further evaluation showed a normal solitary right kidney with no cysts and no abnormalities of the urinary tractus at the renal ultrasound and a normal voiding cystourethrogram. Renal scintigraphy confirmed the congenital solitary kidney with no scars. During the follow-up she developed a profound hypomagnesemia (0.5 mmol/L) and severe extrarenal involvment with hypothyroidy, Crohn's disease and polyarthritis. A surgical renal surgical biopsy was then performed.

Biopsy revealed isolate gomerular cyst which were lined by flat epithelium. The occurrence of glomerular tufts within at least 10 to 20 % of otherwise identifiable cysts. The glomerular tuft within a cyst appeared normal or artrophic and immature. Rare glomeruli were totally sclerotic. The glomerular cysts were isolated without tubular ectasia or cyst. Stroma was not fibrotic and presented no inflammation. The patient was diagnosed with

glomerulocystic kidney disease followed by genetic testing and she was found to be heterozygous for a complete deletion of hepatocyte nuclear.factor-1 HNF-1 gene confirming a diagnosis of renal cysts and diabetes syndrome (RCAD). This result was followed by the discovery of an early-onset diabetes in this patient. The genetic testing of her family is in process.

CD133 Positive Stem Cells In Hepatocellular Carcinoma

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Background: Recent data support the existence of cancer stem cells in some and, perhaps, all cancers. Cancer stem cells may arise from tissue-specific stem cells that arenecessary for the continuous renewal and remodelling of normal tissues. While cancers are a heterogeneous mix of cells at various stages of differentiation, it is thought that undifferentiated tumour-specific stem cells acquire the transforming mutations necessary for tumourigenesis.In the present study, we have investigated thepotential role of stem cells in the genesis of hepatocellular carcinoma. We analyzed CD133, a stem cell marker, in hepatocellular carcinoma arising in cirrhotic and non-cirrhotic liver with comparison to normal liver.

Methods: We examined the expression of CD133 in carcinomas arising in both cirrhotic and non-cirrhotic livers as well as in fibrolamellar carcinoma and normal liver. We chose to use immunohistochemical studies as they may provide key information as to the spatial and temporal relationship between CD133-expressing stem cells and tumour cells. These studies have the potential to identify novel contributory role of precursor/stem cells in hepatocellular carcinoma.

Results: Normal liver demonstrated rare presence of CD133 positive cells. In the cirrhotic liver, however, the number of CD133 cells was increased. A similar increase was seen in the neoplasms. Interestingly, CD133 were mostly localized in the nuclei. Furthermore, among the hepatocellular carcinomas, nuclear staining was pronounced in fibrolamellar carcinomas.

Conclusion: Our findings indicate increased number of stem cells in hepatocellular carcinoma suggesting an important contributory role of these precursor cells in neoplastic proliferation.We plan to investigate the exact significance of these stem cells by (a) extending this study and including a large number of cases, and (b)isolating these CD133-expressing stem cells from hepatocellular carcinomas in order to determine the tumour-initiating capacity. These findings may identify novel cellular targets for therapy.

Adenocarcinoma Arising in Cystic Rectal Duplication, Preceded By Retrorectal Dermoid Cyst: A Case Report

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Background: Retrorectal developmental cysts are congenital lesions. Morphologically these are classified as enteric cysts, epidermoid cysts, dermoid cysts and neurenteric cysts. Enteric cysts include cystic rectal duplication and tailgut cysts. Intestinal cystic duplication can occur at any site in the gastrointestinal tract with the majority in the ileum. Retrorectal cystic duplication is a rare congenital anomaly (5% of all intestinal duplications) which commonly presents in adulthood and more in females. Most cystic rectal duplications usually present with mass effect, bleeding or infection. An estimated 12% incidence of malignant transformation in cystic rectal duplication has been reported.

Case Report: We report a case of a cystic rectal duplication in a 34-yearold male who presented with recurrent presacral abcesses and cutaneous fistulae. Trans-sacral excision was performed, revealing a red-brown cystic lesion. Sections demonstrated a unilocular cyst lined by colonic, respiratory and gastric mucosa with underlying organized thick muscle bundles and nerve fibres. There was invasive adenocarcinoma arising from the mucosa and infiltrating

the underlying muscle. Of significance, the past history included the excision of a retrorectal cyst seven years previously. Review of these slides showed a dermoid cyst with no evidence of malignancy.

Conclusion: This case highlights an interesting, and we believe, a not previously reported association between a retrorectal dermoid cyst and a duplication cyst, which in this patient has undergone malignant transformation.

Association of Urothelial Carcinoma with a Mutated Form of BK Polyomavirus: A Molecular Pathology Study

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Background: Following organ transplantations, immunosuppression carries the risk of a higher risk of developing cancer. BK polyomavirus re-activation is common after renal transplantation. We report a 65-year-old female patient with an urothelial carcinoma in her renal allograft where the tumour was associated with a mutated genotype of BK polyomavirus.

Design: The tumour was investigated by histopathology, immunohistochemistry, electron microscopy, and molecular biology methods. PCR of the tumour was performed with primers specific for the BK polyomavirus and the amplified product was digested with the BamH1 restriction enzyme. The amplified DNA fragment was also sequenced.

Result: Following nephrectomy, a transitional cell carcinoma was found in the pelvis of the renal allograft. Immunohistochemistry showed that all tumour cells were strongly positive for polyomavirus. Electron microscopy showed 42 nm viral particles in the nuclei of the tumour cells. PCR of the micro-dissected tumour generated a DNA fragment which could not be cut by the BamH1 restriction enzyme. DNA sequencing of the PCR product showed a mutation in the BK polyoma viral genome: GGATTC instead of the wild type GGATCC sequence.

Conclusion: Our results indicate that the BK polyomavirus may be present within tumour cells of urothelial carcinoma developing in allografted kidney, and might participate in this particular form of tumorigenesis. Furthermore, this report is the first demonstration of an association between a mutated form of BK polyomavirus and a human malignant tumour.

Do Her2 Overexpressing Breast Cancers Have a Distinctive Morphologic Appearance?

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Objective: This study aimed to characterize the clinicopathologic features of HER2 positive (HER2+) breast cancers and to determine if these tumours have a distinct morphologic appearance.

Methods: A consecutive series of 51 HER2+ and 50 HER2 negative (HER2-) grade II or III breast cancers were reviewed. The following parameters were assessed: grade (including individual components), invasive tumour necrosis, lymphovascular invasion (LVI), lymphocytic infiltration (LI), apocrine differentiation, pushing vs infiltrative margins, presence and grade of DCIS, comedo necrosis in DCIS, extensive intraductal component (EIC), multifocality, lymph node (LN) involvement and number of LNs involved. Statistical analysis was performed using χ^2 or Fisher exact test with a significance level of $P<0.05$.

Results: HER2+ tumours were significantly more likely to be ductal, no special type (100% vs 88%) and to show apocrine differentiation (67% vs 28%), infiltrative margins (90% vs 70%), a mild or moderate LI (95% vs 57%), DCIS (90% vs 68%), high grade DCIS (78% vs 41%), comedo necrosis (92% vs 56%) and EIC positivity (32% vs 10%). Of the HER2+ tumours 55% were ER-/PR- and 45% ER+/PR+/low (triple positive). There was no significant difference between tumour size, age of patient, LVI, multifocality or LN involvement between the two groups.

Conclusion: HER2+ tumours show a distinctive morphology. In particular, they are significantly more likely to show apocrine differentiation. This finding warrants further exploration.

Reduced Expression of WT-1, ER, and PR in Uterine Leiomyosarcoma Compared to Benign Leiomyoma: A Clinicopathological Study

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Objectives: WT-1 protein, a transcription factor essential for development of the genitourinary system, is normally expressed in uterine myometrium. We explored the expression of uterine smooth muscle markers (WT-1, ER and PR) among benign and malignant uterine smooth muscle tumours (USMTs).

Methods: Fifty-two cases of USMTs including leiomyosarcomas (LMS, n = 15), STUMP (n = 4), leiomyoma variants (LV, n = 14) and benign leiomyomas (BL, n = 19) were retrieved from the laboratory database. A tissue array was created for each group of tumours. Immunohistochemical staining was performed for WT-1, ER and PR and each case was given a value of 0 to 100% positive cells. Comparison of numerical data was performed with the Kruskal-Wallis test. Correlation with pathological and clinical outcome parameters was done in all cases.

Results: All three markers studied were found to have statistically significant lower expression in LMS compared to BL, while showing some minor reduction of expression in STUMP and LV categories. The median percentage expression of WT-1 (2% vs. 65%; p =.014); and both ER and PR (10% vs. 100% ; p<.001) showed significant difference in LMS vs. BL cases.

Conclusions: Leiomyosarcomas show reduced nuclear WT-1, ER, and PR immunostaining compared to benign leiomyomas and this feature may aid in the differential diagnosis of difficult cases. Since these markers are seen in normal myometrium, the significantly lower expression of these markers in leiomyosarcomas may represent de-differentiation of malignant tumour cells.

Granulomatous Leptomeningitis: A Case Report

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Adalimumab (Humira) is medication that is composed of a human monoclonal antibody that binds to tumour necrosis factor alpha (TNF-α), thereby modulating the immune system. It is widely used in the treatment of inflammatory conditions such as rheumatoid arthritis. We report a case of a 56-year-old female with a 5-year history of rheumatoid arthritis, treated with adalimumab. The adalimumab was stopped after 5 months due to the development of bilateral paresthesias of the lower extremities. Two months following the cessation of treatment, the patient presented to the emergency department with personality changes, inappropriate vocalizations, occasional aphasic staring spells, and problems with inattention and balance. Within days the patient deteriorated, being unable to perform most activities of daily living without assistance. Neuroimaging showed bifrontal vasogenic edema and leptomeningeal enhancement. Microscopic examination showed multiple necrotizing granulomas with scattered multinucleated giant cells within the leptomeninges and a dense chronic inflammatory cell infiltrate within the subarachnoid space. Multiple sections stained with gram stain, GMS, PAS and Ziehl-Neelsen revealed no infectious organisms. CSF gram stain, acid fast stain, 16s rRNA PCR and culture were negative. The patient was treated with high doses of corticosteroids and rapidly improved. Four months following treatment, the patient has recovered with no significant neurological deficits.

Post-transplantation Lymphoproliferative Disorders: An Etiomorphologic Study

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Objectives: Posttransplantation lymphoproliferative disorders (PTLDs) are a heterogeneous group of diseases that are frequently associated with EBV. In this study, we investigated the sites of occurrence and EBV status in relation to the subtypes of PTLD.

Materials and Methods: We reviewed the surgical pathology files (1997-2009) of 53 cases of PTLD in 47 consecutive

patients with solid organ transplantation. EBV status by in-situ-hybridization (EBV-ISH) was studied.

Results: In the 47 patients (11 children, 36 adults, age mean=41, range 2-71, F:M=2:3) with solid organ transplants (19 kidneys, 10 livers, and 9 each of hearts and lungs), two (4%) cases developed early lesions of PTLD with plasmacytic hyperplasia, 19 (36%) cases with polymorphic (PM) PTLD and 28 (53%) cases had monomorphic (MM) subtype, of predominantly diffuse large B-cell lymphoma, and 4 (7%) cases of Hodgkin lymphoma (HL). The aerodigestive tract represented the largest involved group (19 cases 36%: GI=11, respiratory tract=8), with 8 cases (42%) of PM and 11 (58%) MM. This was followed by involved lymph nodes 17 cases (32%) and liver 9 (17%). Six cases had multiple organ involvement. In 35 cases tested by EBV-ISH, 26 (74%) were found positive (17 MM-HL, 9 PM) and 9 (26%) were EBV-negative (7 MM, 2 PM). The EBV status was compared in PTLD subtypes, and found to be statistically insignificant (p value > 0.05).

Conclusions: This study confirms the heterogeneity of PTLD and the involvement of multiple anatomic sites. The high frequency of occurrence of PTLD in the aerodigestive tract is interesting, and may be related to the immunologic reactions in mucosa associated lymphoid tissues (MALT) in these sites. EBV continues to be an important etiologic agent in the development of PTLD. The emerging EBV-negative PTLD cases may warrant research into other etiologic agents and different pathogenetic mechanisms.

Frequency of Failure to Correct for Type I Statistical Errors In Pathology Papers and Its Effect on Publication Citations

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Objective: Type I statistical errors occur if the alternative hypothesis is accepted but the null hypothesis is actually correct. By convention the acceptable rate of this error is set at 5% (alpha = .05). However, when multiple comparisons are presented, although each comparison may have an alpha = .05, the overall risk of committing a type I error increases proportional to the number of tests. In our experience this fact has been widely ignored in the pathology literature. The purpose of this study was to document the frequency of these type I errors in the pathology literature and then to test if the presence of such an error is correlated with the subsequent number of citations the paper receives.

Methods: We reviewed papers from seven pathology journals published in 2003. We then used Google Scholar to determine the number of citations of these papers over the following five years.

Results: Of 800 papers, only 37 (4.6%) presented multiple comparisons, and of these 21 (2.6% of total) did not correct for multiple comparisons. The number of citations of studies correcting for type I error (11.3) and those not correcting (11.1) was not statistically different (unpaired t-test, $p = .958$).

Conclusions: Although relatively few pathology papers present multiple statistical comparisons, of those that do, a majority do not correct for multiple comparisons. To the extent that the number of citations can be used as a measure of the overall impact of a paper, there is no evidence that papers failing to correct for type I error are viewed differently. This may have significant implications for the possible entry of spurious findings into the pathology literature.

Endoscopic Ultrasound Guided Fine Needle Aspirations of the Pancreas and Other Organs: Five-Year Experience in the Province of Manitoba

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Objective: Endoscopic ultrasound guided fine needle aspirations (EUS FNA) combining endoscopic diagnostic ultrasonograpy and fine needle cytology was introduced in 1992. Manitoba started using this diagnostic method in 2005. EUS FNA has since been used in the diagnoses of a variety of pathological conditions, in particular pancreatic lesions. The goal of this study was to use clinical pathologic correlations in the assessment of diagnostic accuracy, the learning curve and reasons for discordance.

Methods: All EUS FNAs were done by one of the authors, a gastroenterologist with special training in endoscopic

diagnostic ultrasound. The FNAs were performed using an Olympus video endoscope equipped by a curvilinear high frequency transducer and utilizing a conventional 22-gauge multi-use needle system. A cytotechnologist in the endoscopy suit using Quick-Diff stain pre-screened aspirates. Diagnosis was rendered by a cytopathologist based upon PAP stain, H&E and immunohistochemistry. Surgical and clinical follow up were sought in all cases and analyzed using parametric and non-parametric statistics.

Results: Approximately 400 patients underwent EUS FNA procedure during 2005–2009. Roughly half of them were aspirates from a variety of pancreatic lesions. Solid lesions showed a much higher rate of accuracy and concordance than cystic lesions. Approximately 20% were unsatisfactory, 30% were negative, 20% were atypical, and 30% were suspicious or malignant. In cases where surgery was done, approximately 55 % were carcinomas, 10% pancreatic endocrine tumours, 7% mucinous neoplasms and 28 % others. Unsatisfactory and non-diagnostic results declined, as expected, with increasing experience.

Conclusion: EUS FNA is a valuable minimally invasive diagnostic procedure requiring close cooperation of endoscopist and cytopathologist.