

## **ANATOMICAL PATHOLOGY ORAL PRESENTATIONS**

### **APO 01: A digital collection as substitute for the pathology museum**

**Jane Yeats, Lorna Martin. University of Cape Town**

Up until the first half of the 20th century, pathology museums played a hugely important role in medical education. This role has steadily declined, some reasons being the easy availability of patients for clinical teaching in large referral hospitals, decreased teaching time for anatomical pathology, colour photography textbooks, and simply the space and money that museums require to operate.

UCT has several museum collections containing thousands of specimens, a small proportion of which are still actively used for teaching, but most of which have been effectively mothballed. These collections hold 80 years worth of material, including pathology that is rare in the 21st century, specimens reflecting the recent history of medicine (such as early heart transplantation work) and whole organ specimens that are now very seldom available due to modern pathology processing. They contain the “classic cases” for teaching undergraduates as well as the “canaries” for postgraduates. The specimens will become ever more valuable since they are ever less likely to be added to in the face of local economic and legislation hurdles and following the Alder Hey inquiry.

To increase the accessibility to and use of the collections by contemporary students (who are known to prefer computer-based learning), we have initiated a web-based digital pathology collection at [www.digitalpathology.uct.ac.za](http://www.digitalpathology.uct.ac.za). This approach is in line with the change in medical education towards problem-based self-directed learning. High quality digital images with electronic catalogues are gradually being transferred to the site, as well as supplementary teaching material. UCT will be able to make its museum resources available to students at other African universities, and share in the global trend of on-line learning.

### **APO 02: The Groote Schuur Hospital Breast Clinic - Old Traditions and New Challenges**

**Sharon Fenwick, Cytotechnologist, NHLS at Groote Schuur Hospital.  
Genevieve Warner Learmonth, Histopathologist/Cytopathologist, NHLS at Groote Schuur Hospital and University of Cape Town.**

The incidence of breast cancer in the general population is 1:12. The incidence of benign breast lumps is difficult to calculate. Clinical staff in the Breast Clinic is required to distinguish between the two. In 1951, a “Breast Clinic” was founded at GSH, to review histopathology results, recall patients and check their progress. The existing GSH Breast Clinic as it is known today, was formed in 1982 with the objective of providing a “real time “on- site Fine Needle Aspirate (FNA) cytology diagnosis and a multidisciplinary approach to treatment and care. Triple assessment with correlation of clinical impression/mammogram/cytology, is strictly practiced. Comparison of the outcomes of the first 1,500 cases during the period 1982-1985 is compared with the outcomes of 1,500 consecutive cases from January 2008 –March 2009. Histopathological correlations with Cytology diagnoses showed that the incidence of “false positive“ cytology diagnosis for malignancy remained fairly constant (9/7). The number regarded as “suspicious” decreased (150/84); thereby enabling clinicians to manage patients more confidently. The number of aspirates classified as “inadequate” increased (292/379). The problem of “inadequate” samples in FNA practice needs to be addressed to avoid loss of confidence in and maintain reliance on cytological diagnosis as the cornerstone of Triple Assessment in hospital outpatient management. FNA of breast lumps with “real time”

cytology diagnosis has proved to be an accurate, inexpensive, quick and effective diagnostic tool for the triage of patients in an overburdened Breast Clinic.

**APO03: Magnetic Resonance Imaging-pathologic correlation in Neurotuberculosis: evidence for the need to revise morphologic principles and terminology.**

**Dan Zaharie, Richard Hewlett. Division of Anatomical Pathology, Department of Pathology, Stellenbosch University and NHLS Tygerberg Hospital**

**BACKGROUND.** Neurotuberculosis is a major field of study at Tygerberg Hospital and the University of Stellenbosch Medical School, Cape Town, ongoing for the past 30 years. Fields of interest include epidemiology, drug therapy, clinical diagnosis & management, imaging and neuropathology.

**DESIGN.** All biopsy and autopsy material is recorded on the neuropathology database, together with the images whenever these are obtainable. ZN, PAS, methenamine silver & reticulin methods are routine. MRI technique includes T2, FLAIR, and contrast-enhanced T1WI in 3 planes. After image correlation, the final histologic diagnosis attempts to settle the category of the necrotising process, ie. gummatous or caseous, or mixed. In autopsy material, the disease process is also defined as basal or focal, proliferative or exudative.

**RESULTS.** Correlative imaging and pathology has unequivocally yielded the fact that in focal lesions, the necrotising process is predominantly MR T2 hypointense (non-enhancing), histologically gummatous, ZN negative. By contrast, focal T2 hyperintense lesions ie. pseudoabscess, are invariably caseous, usually ZN positive. However admixtures of both types of reaction typically coexist in tuberculous meningitis.

**CONCLUSION.** The demonstration on MRI, of two distinct types of mass lesion in CNS tuberculosis, has necessitated revision of the histologic definition of the necrotising process, and the use of more exact terminology, ie. gummatous versus caseous. Although the presence of coagulative necrosis of inflammatory granulation tissue, ie. gumma, is always apparent on the H&E preparations, this reaction is best shown with the reticulin method. Practical application of these principles includes the now clinically-accepted differences in response to treatment of TB gumma and pseudoabscess, the relevance of ZN negativity or positivity, and the recent elucidation of the role of apoptosis in caseous necrosis. The influence of HIV in the evolution of the necrotising process needs to be elucidated.

**APO04: The wnt signaling pathway and downstream effects in solid pseudopapillary tumour of the pancreas.**

**Hue-Tsi Wu, Bilqees Cassiem. Nafiesa Allie, Ahmed Motala, Dhirendra Govender. Division of Anatomical Pathology, University of Cape Town and NHLS Groote Schuur Hospital**

**AIM:** Solid pseudopapillary tumour of the pancreas (SPTP) is a rare neoplasm that may pose diagnostic difficulties. Beta-catenin is an intracellular protein present as a membrane bound form complexed to E-cadherin and also as a cytoplasmic form that is ubiquitinated and degraded in the proteasome. Nuclear translocalisation of beta-catenin activates downstream proto-oncogenes such as cyclin D1 and c-myc. This study aims to investigate E-cadherin and beta-catenin expression, and to determine the expression of beta-catenin downstream targets, cyclin D1 and c-myc.

**METHODS:** Nine cases of SPTP were studied. Archival formalin-fixed paraffin-wax-embedded tissue sections were stained with antibodies to beta-catenin (17C2), E-cadherin (36B5 and 36/E-

cadherin), c-myc (9E11), and cyclin D1 (SP4) using the Envision kit and diaminobenzidine as chromogen.

**RESULTS:** All nine cases showed nuclear expression of beta-catenin, c-myc, and cyclin D1. Loss of membranous E-cadherin (clone 36B5) expression was seen in all cases. In addition, the 36/E-cadherin clone against the cytoplasmic domain of E-cadherin showed nuclear immunoreexpression in all cases.

**CONCLUSION:** This study confirms that nuclear beta-catenin expression and loss of membranous E-cadherin (with nuclear localisation of the cytoplasmic domain of E-cadherin) is a unique finding in SPTP that is a potential diagnostic aid and contributes to the understanding of the oncogenesis of this tumour.

#### **APO05: The Neuropathology of Fetal Alcohol Syndrome: Report of 3 Cases**

**Rebecca D. Folkerth, Department of Pathology, Brigham and Women's Hospital, Children's Hospital, Harvard Medical School, Boston, MA, USA**

**S. Dan Zaharie, Richard H. Hewlett. Division of Anatomical Pathology, Department of Pathology, Stellenbosch University and NHLS Tygerberg Hospital.**

**Hein J Odendaal, Department of Obstetrics and Gynecology, Stellenbosch University and Tygerberg Hospital.**

The questions facing the pathologist upon examination of the brain of an infant or toddler who has died suddenly and unexpectedly are: 1) is there a brain lesion that explains sudden death?; 2) if not, is there a brain lesion that reflects a systemic process that explains sudden death?; and 3) is there a brain lesion that, if not directly causative, provides insight into the pathogenesis of sudden death? Brain lesions responsible for sudden death in early life include traumatic hemorrhages and infectious processes. Brain lesions indicative of a systemic process include white matter pathology that complicates inborn errors of metabolism. Certain microscopic brain lesions with the light microscope in infants dying of the sudden infant death syndrome (SIDS) are likely secondary and provide potential clues to its pathogenesis, including diffuse gliosis of the cerebral white matter and brainstem and periventricular leukomalacia. These lesions suggest a role for perinatal and/or chronic/episodic hypoxia-ischemia in SIDS. The light microscope may also reveal subtle lesions in SIDS that suggest causative mechanisms. Hypoplasia of the arcuate nucleus at the ventral surface of the medulla in SIDS cases is known to be associated with wider brainstem neurotransmitter deficits related to cardiorespiratory control. The arcuate nucleus encompasses the putative respiratory chemosensitive fields involved in chemosensitivity to carbon dioxide. In toddlers (1-5 years), abnormalities of the hippocampus are associated with sudden death, and include gross asymmetry and multiple microdysgenetic features. A lethal, sleep-related seizure originating within an epileptogenic focus in the hippocampus is postulated. Taken together, this information indicates that a systematic examination of the brain is essential in the evaluation of sudden death in infants and toddlers at autopsy.

#### **APO06: Morphometric analysis of endomyocardial biopsy specimens from the South African Arrhythmogenic Right Ventricular Cardiomyopathy Registry**

**N Morse, NHLS, Groote Schuur Hospital, University of Cape Town.**

**H Wainwright, NHLS, Groote Schuur Hospital, University of Cape Town.**

**B Mayosi, Department of Medicine, Groote Schuur Hospital, University of Cape Town.**

**N Hendricks, Department of Medicine, Groote Schuur Hospital, University of Cape Town.**

**BACKGROUND:** Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is a genetic disease of autosomal dominant inheritance. It is characterised by fibro-fatty replacement of the right ventricular myocardium with associated ventricular dilatation, arrhythmias, ECG abnormalities and sudden cardiac death. One of the major criteria for the diagnosis of ARVC/ D is the presence of fibrofatty replacement of the myocardium in endomyocardial biopsy (EMB) specimens. EMB diagnosis is inherently difficult because of the focal nature of the disease and the septum being frequently sampled. Cardiologists rely on clinical criteria to make the diagnosis. Various authors have made suggestions regarding quantitative values diagnostic of ARVC with varying sensitivities and specificities.

**AIM:** To compare morphometric values of EMB biopsy specimens to the qualitative histologic findings and clinical diagnosis.

**METHODS:** We performed morphometric analysis of EMB specimens from 33 patients in the South African ARVC registry, using the Nikon Coolscope digital microscope and software. The clinical diagnosis was established using a diagnostic panel set out by the 1994 task force criteria. Using the quantitative values set out by various authors, we compared the results of the morphometry with the clinical diagnosis.

**RESULTS:** The results showed that Angelini's value of >3% adipose tissue had the best sensitivity and specificity (66 and 83% respectively) with regards to the clinical diagnosis. Witcher's criteria of fibrofatty replacement of the myocardium showed 55% sensitivity and 66% specificity and this is similar to their original published outcomes. Residual myocytes of <60% as proposed in the new task force criteria had a 59% and 50% sensitivity and specificity respectively.

**CONCLUSION:** We are able to conclude that experienced pathologists with knowledge of the diagnostic criteria required for ARVC/D had a sensitivity of 83% and a specificity of only 66% compared to the morphometric values.

## **APO07: Biomarker identification in formalin fixed paraffin embedded tissue using proteomics methods**

**Richard Naidoo, Division of Anatomical Pathology, UCT/NHLS, Groote Schuur  
Anushka Moothoo-Padayachie, Patrick Govender. Division of Biochemistry, UKZN  
Michael Locketz, Dhiren Govender. Division of Anatomical Pathology, UCT/NHLS, Groote Schuur**

**PURPOSE OF STUDY:** The aim of this study was to investigate the protein profile in a cohort of gastric carcinomas using a novel proteomics method.

**METHODS:** Novel molecular technologies have had a major influence in both research and diagnostic medicine. This paper addresses the use of a protein isolation method using proteomics technology as a means of identifying potential biomarkers. A cohort of formalin fixed paraffin embedded gastric carcinomas and corresponding normal tissue were used in this study (N=10: Pilot study). Sections (5µm) were cut, de-waxed and hydrated. Normal and tumour tissue was scraped and subjected to ethanol/formic acid extraction procedure. The protein extracts were analysed using MALDI-TOF/MS and the mass spectra generated was evaluated by Principle Component Analysis (PCA). PCA was performed using the ClinproTools software for biomarker detection and evaluation.

**RESULTS:** Our preliminary findings show significant differences in the MALDI-TOF/MS mass spectra between the normal and corresponding tumour samples.

**CONCLUSIONS:** Proteins can be isolated from formalin fixed paraffin embedded tissue using the methods adopted. Although the procedure requires further optimization there are obvious differences in the protein profiles seen between the tumour and corresponding normal tissue. This data will be further analysed for the identification for potential biomarkers after correlating the clinical and pathological data.

## **APO08: Maternal Death: Unusual, unexpected and rare causes**

**CM Dittrich, HC Wainwright. Division of Anatomical Pathology, University of Cape Town and NHLS Groote Schuur Hospital.**

**INTRODUCTION:** Maternal deaths are a major concern of the Department of Health and are notifiable in terms of the Health Act. The 2008 Guidelines of the National Committee on Confidential Enquiries into Maternal Deaths classifies disease entities causing maternal death into: no obstetrical cause, medical and surgical disorders, non-pregnancy related infections, ectopic pregnancy, miscarriage, hyperemesis gravidarum, pregnancy-related sepsis, obstetric haemorrhage, hypertensive disorders of pregnancy, anaesthetic complications, embolism, acute collapse and unknown.

**AIM:** To audit maternal deaths in our institution, to compare primary and antecedent causes to national data, and to identify unusual, unexpected and rare causes of maternal death.

**METHOD:** An audit of autopsies performed in the Division of Anatomical Pathology at UCT from 2004 to date was done using the post mortem register. All maternal deaths were identified. These were stratified according to the cause of death listed in guidelines for completing the maternal death notification form (3rd edition, 2008). Other parameters included age, gestation, HIV status, antenatal care and method of delivery. Forensic autopsies (anaesthetic complications, no obstetrical cause) were excluded.

**RESULTS:** A total of 40 autopsies were performed including 8 partial and 32 full autopsies.

CAUSE OF DEATH	NUMBER OF CASES	PERCENTAGE
Hypertensive disorders of pregnancy	16	40
Non-pregnancy related infections	8	20
Medical and surgical disorders	5	12.5
Pregnancy-related sepsis	5	12.5
Embolism	2	5
Acute collapse	2	5
Hyperemesis gravidarum	1	2.5
Obstetric haemorrhage	1	2.5
Ectopic pregnancy	0	0
Miscarriage	0	0
Unknown	0	0

35% were HIV-positive, 10% negative and 55% unknown, mirroring booking status for antenatal care. There were five cases with incidental parasitic infestation.

**CONCLUSION:** Our figures are on par with national data with respect to the major causes of maternal death but hypertensive conditions outnumber non-pregnancy related infections.

## **APO09: Autofluorescence on Papanicolaou stained smears: a rapid ancillary diagnostic technique**

**Greta Neethling, Mercia Louw, Pawel Schubert, Colleen Wright. Division of Anatomical Pathology, Department of Pathology, Stellenbosch University and NHLS Tygerberg Hospital**

**AIM:** To confirm the autofluorescent (AF) properties on Papanicolaou (PAP) stained smears of pathogenic organisms, particularly those seen in immune compromised patients. To optimize the PAP stain technique.

**METHOD:** Fifty-one Smears on whom a pathogenic organism had been identified were retrieved from the archives of the Cytology Laboratory, NHLS, Tygerberg Hospital, and the Pap stained smears were examined with blue excitation filter (450nm).

**RESULTS:** Organisms which were subjected to fluorescent microscopy included fungi, (Candida+, Phycomycosis+, Pneumocystis+, Cryptococcus+, Histoplasmosis+, Trichophyton+) bacteria, (M. tuberculosis+, M. leprae+, M.bovis BCG+, Chlamydia+, Actinomyces+) protozoa, (Amoeba+, Trichomonas+) viruses, (Measles+, Molluscum+, HPV-, Herpes-, CMV-) and other parasites. (Echinococcus+, Schistosoma-) These organisms showed various degrees of green to yellow fluorescence, highlighting the cell walls. The age of the specimens did not play a role in the strength of AF, as many cases were more than 10 years old.

We confirmed that this technique is only effective on smears stained with the conventional, regressive PAP (CP) method, and not with the modified, rapid PAP (RP)

AF is a rapid diagnostic modality that is ideal for use in immunocompromised patients as it identifies three of the most common pathogens seen in AIDS patients i.e. Pneumocystis, Cryptococcus and Mycobacterium. The PAP stained smears are preserved ideal for limited material. The advent of inexpensive LED fluorescent microscopes makes this technology affordable in resource-limited countries.

**CONCLUSION:** This is an ideal ancillary test to perform on conventional PAP stained smears from patients in whom pathogenic infections are suspected. A broad spectrum of organisms may be identified with this simple technique, without destroying the original smear.

## **ANATOMICAL PATHOLOGY POSTER PRESENTATIONS**

### **APP01: Results of a probe into perceptions on joint FSASP-SMLTSA congresses.**

**Erich Raubenheimer, FSASP and University of Limpopo.**

After the first joint FSASP-SMLTSA congress in 2009 in Durban, the FSASP distributed a questionnaire amongst members of the IAP, SAACB and SASH on the desirability of biennial joint congresses. The response rate was poor (32 responded which is less than 10% of the total membership). Indications are that 94% of respondents support the concept of a biennial joint congress. Only two members indicated that they would not attend future joint congresses. The reasons given relate mainly to the perceived quality of some papers. Valuable comments included a desire for more educational presentations, restriction of the congress to weekends, elimination of parallel sessions with overlapping interests, succinct opening ceremonies and less noise during the banquet.

### **APP02: Histomorphometry of metabolic bone diseases: A report on 150 cases.**

**Erich Raubenheimer. FSASP and University of Limpopo.**

**Denise Potgieter. Little Company of Mary Hospital.**

**Herman Joubert. NHLS and University of Limpopo.**

Metabolic bone diseases generally manifest at late stage with severe skeletal debilitation. The purpose of this study was to record the histological- and histomorphometric features of 150 cases of metabolic bone diseases diagnosed at the University of Limpopo over a 25 year period. All patients were subjected to two cycles of tetracycline administered 12 days apart. Trans-cortical iliac crest biopsies were taken, processed for non-demineralized sections and stained with H&E-, Picrosirius- and Von Kossa techniques. An unstained section was examined with UV illumination in order to assess the extent of tetracycline incorporation. The morphology of the bone was studied and the

cortical- and trabecular bone content, volume of osteoid, osteoclast distribution and mineralization activities measured and compared with reference values. Osteopetrosis (n=2), hypertrophic rickets (n=83) and hypophosphatemic rickets (n=4) showed osteoid covering more than 50% of bone surfaces. Hypodynamic bone with a reduction of all measurable parameters was characteristic for senile osteoporosis (n=7), hypothyroidism (n=3), hypogonadism (n=2) and juvenile osteoporosis (n=1). Elevated osteoclast activity was the hallmark of secondary hyperparathyroidism (rickets and high turnover renal osteodystrophy) and malignancy induced osteopaenia. The activities of osteoblasts and osteoclasts were found to be delinked in the latter. Follow-up biopsies were pivotal in quantifying responses to therapeutic regimes. This study demonstrates the value of histomorphometry in the diagnosis and management of metabolic diseases of bone.

**APP03: Chronic lymphocytic leukaemia and post-herpetic cutaneous reaction: an isotopic phenomenon.**

**Christine Crause, Jurg E Dinkel. Department of Anatomical Pathology, University of Pretoria/NHLS.**

**John Moche. Department of Dermatology, University of Pretoria.**

We report a case of a 70 year old male patient who was diagnosed with chronic lymphocytic leukaemia (CLL) in August 2009. He had three cycles of Chlorambucil. In January 2010, he presented with multiple papules of the skin on his forehead, neck and back. He recently had an episode of herpes zoster infection affecting the skin in the trigeminal nerve distribution. Biopsies were taken from the forehead, nose and back area. Histological examination confirmed the presence of a dense lymphoid infiltrate, which extended into the papillary dermis. The neoplastic cells ranged from smaller and 'mature' to somewhat larger lymphoid cells, predominantly representing CD20 positive B-cells with variable CD5 and focal CD23 expression. The phenotype was compatible with CLL (chronic lymphocytic leukaemia). In addition, features of granulomatous inflammation involving occasional hair follicles as well as granuloma formation within the dermis were noted. The stains for infective etiological agents including Ziehl Neelsen and PAS stains, proved negative. No histological features of a current herpes infection were seen and the HSV (herpes simplex virus) immunohistochemical stain was negative. Although the presence of a granulomatous reaction has been described in patients with Non Hodgkin's Lymphoma, they are rarely observed in CLL. In the literature, granulomatous post herpetic cutaneous reactions have been reported. The pathogenesis of post herpetic cutaneous reactions is now better understood since the concept of an isotopic response was introduced. Isotopic response is defined as the occurrence of a new skin disease (isotopic disease) at another, unrelated and already healed site of the skin. In this case, presentation the primary disease was the herpes zoster infection. The virus altered the skin immune response and induced hyperreactivity towards various tissue antigens with the formation of granulomas. In addition, infiltration by neoplastic cells of B- CLL was present.

**APP04: Simultaneous medullary and follicular carcinoma of the thyroid: a case report.**

**Paula Eyal. NHLS TAD**

A case of simultaneous medullary and follicular carcinoma of the thyroid in a 54 year old male is described. No clinical details were provided by the clinicians and the specimen was received identified only as "thyroidectomy". On macroscopic examination, two lesions were noted, identified on histology as separate foci of medullary carcinoma and follicular carcinoma of the thyroid. Both

lesions showed typical histological features and both were demonstrated with appropriate immunohistochemical stains. No admixture of the two tumours is present, and the diagnosis is therefore that of a double malignancy. This has been very infrequently described in the literature, and this case report serves to provide further evidence as to the occasional simultaneous occurrence of these two tumours in the same thyroid.

#### **APP05: Mucinous tubular and spindle cell carcinoma of the kidney.**

**Gerhard van der Linde, NHLS, SA**

Mucinous tubular and spindle cell carcinoma (MTSCC) is a less common pattern of renal cell carcinoma (RCC) characterized by an epithelial-tubular proliferation admixed with spindle cell areas and a bubbly, myxoid stroma. This tumour was initially referred as an unusual renal carcinoma with prominent spindle cell change, RCC with loop of Henle differentiation, low-grade myxoid tumor and low grade collecting duct carcinoma. In 2004, it has been recognized as a distinct entity of RCC in the 2004 World Health Organization tumor classification. Since then, several dozen of MTSCCs have been reported with additional complementary morphology features, immunohistochemical profiles, and cytogenetic changes that have further clarified its clinicopathological features. It was initially thought to be a "low-grade" renal cell tumor because of its bland nuclear features and indolent behavior. However, it has become clear that MTSCC has a histological spectrum ranging from low to high grade that includes, rarely, sarcomatoid differentiation. In addition to nodal metastasis, distant metastasis and death of patients have been described.

I herewith present a case of MTSCC and summarize the current knowledge regarding histological and immunohistochemical features, differential diagnosis, and prognostic factors.

#### **APP06: Internal morphology of ameloblastomas: a study of 24 resected specimens.**

**SP Ngwenya, University of Limpopo.**

**OBJECTIVES.** The objectives of this study were to describe the internal macroscopic architecture of resected specimens of ameloblastoma and to correlate the findings with radiographs and microscopic features.

**STUDY DESIGN.** Resection specimens of 24 ameloblastomas were retrieved from the files of the Department of Oral Pathology at the University of Limpopo. The neoplasms were sectioned in parallel slices and the macroscopic features recorded and each slice was radiographed and sampled for microscopic examination. The macroscopic features were correlated with respective microscopic and radiological appearances.

**RESULTS.** Twenty-three ameloblastomas affected the mandible and 1 the maxilla and measured between 3.3 and 20 cm in greatest diameter. Six cases were unicystic, 2 of which showed incomplete septae both of which presented multilocular on radiographs. Intracystic proliferations were present in 15 cases. These proliferations showed macroscopic features of either small or large nodules with or without the formation of confluent plaques, focal papillary lesions, or multinodular masses that protruded into the cystic cavities. Microscopically these proliferations were characterized by foci of inflammation or plexiform or solid epithelial proliferations, one of which showed a focus of carcinoma in situ, adenomatoid differentiation and another osteodentin deposits. Seven cases had foci of stromal desmoplastic change, one of which exhibited mineralized deposits resembling bone.

CONCLUSIONS. The assessment of the cystic nature of ameloblastomas on 2-dimensional radiographs is inaccurate. Intraluminal proliferations, in situ carcinomatous change, adenoid differentiation, stromal osteodentin, and bone deposits and desmoplasia were found to be focal rather than generalized phenomena in resection specimen of ameloblastoma.

### **APP07: Oesophageal Cancer in South Africa: Gene Copy Number Analysis uncovers some candidate genes**

**Jacqueline Brown, National Health Laboratory Service**

**Rob Veale, University of the Witwatersrand**

**Andrej Stepien, Pascale Willem, National Health Laboratory Service**

Oesophageal squamous cell carcinoma (OSCC) has an unusual epidemiological pattern with areas of high prevalence in the Asiatic belt and some regions of Africa. In South Africa, the incidence in the Eastern Cape is one of the highest in the world. The molecular pathology of this disease remains unresolved. SNP (single nucleotide polymorphism) array technology provides a high resolution technique to determine DNA copy number imbalances across the whole genome. DNA copy number changes can affect oncogenes and tumour suppressors, contributing to oncogenesis. The aim of the study was to use this approach to identify candidate genes and pathways involved in OSCC oncogenesis.

We performed copy number analysis on 51 OSCC retrospective samples from the Eastern Cape region using Affymetrix 500K Genotyping Arrays. Copy number states of particular candidate genes identified by arrays were verified by fluorescence in situ hybridization (FISH) in a subset of the samples. Expression of a subset of genes was analysed by real time RT-PCR in 5 oesophageal cancer cell lines and 4 fresh OSCC specimens.

In a previous study we detected a common translocation breakpoint affecting chromosome 3p11.2, deletion of the EPHA3 gene situated at this chromosome position was confirmed by DNA copy number analysis. Amongst a number of other common copy number aberrations detected in the OSCC specimens, EPHA3 was found to be deleted in 61.53% of them. EPHA3 is an ephrin A3 receptor tyrosine kinase that has been shown to have both oncogenic and tumour suppressor functionality [1; 2]. In addition, 8q24.21(C-MYC, FAM84B), 11q13.3-13.4 (CCND1, FGF3, FGF4, FGF19, MYEOV, 3q11.2-29(PIK3CA), 20q11.2-12 (EYA2) amplifications and deletion of 3p14.3 (FHIT) were identified.

This study has highlighted some candidate genes involved in OSCC carcinogenesis, many of these genes feed into the Wnt signaling pathway, a key pathway involved in various cancer types.

### **APP08: Microscopic colitis as a missed cause of chronic diarrhoea.**

**Noor Mohamed, Division of Anatomical Pathology, Department of Pathology, Stellenbosch University, NHLS Tygerberg Hospital.**

**Monique Marais, Division of Gastroenterology, Department of Medicine, Tygerberg Hospital.**

**Juanita Bezuidenhout, Division of Anatomical Pathology, Department of Pathology, Stellenbosch University, NHLS Tygerberg Hospital.**

AIM: To determine the prevalence of increased intraepithelial lymphocytes using immunohistochemistry (CD3), in patients with a normal colonoscopy and near normal biopsy.

METHODS: We retrospectively reviewed all non malignant colon mucosal biopsies between 2005 and 2007, reported as normal, chronic inflammation or melanosis coli in patients having a normal

colonoscopy. Immunohistochemistry using CD3 was performed on all mucosal biopsies and an intraepithelial lymphocyte count (IEL) was performed. Cases with an IEL count of  $\geq 20$  IELs per 100 surface epithelial cells were correlated with demographic, clinical and follow up data.

**RESULTS:** Twenty (8.3%) of 241 cases revealed an IEL count of  $\geq 20$ . Six (2.5%) patients were identified as having lymphocytic colitis ( $p=0.0006$ ) of whom 5 patients were missed on initial evaluation ( $p=0.01$ ). Four of these 5 patients were labelled as irritable bowel syndrome. On follow up, 3 of the remaining 20 cases were diagnosed with malignancy (renal cell carcinoma and myelodysplastic syndrome) and 1 case had an unknown primary with multiple liver metastases. Two cases of collagenous colitis having an IEL count of  $<10$  were included in this study. Increase IEL's was not confined to patients presenting with diarrhoea as a primary presenting symptom but was also present in patients presenting with abdominal pain ( $n=7$ ), constipation ( $n=3$ ) and loss of weight ( $n=1$ ). Poor staining of slides and tangential biopsies accounted for misidentification of lymphocytes on Haematoxylin and Eosin.

**CONCLUSION:** IHC using CD3 is of value in identifying and quantifying IELs for the presence of microscopic colitis in patients presenting with diarrhoea predominant irritable bowel syndrome.

#### **APP09: Extracavitary Primary Effusion Lymphoma - A Case Report.**

**Fabio L Crabbia, Mariaan Kruger, Petrus W Van Zijl, Catherine A Beukes, Jacqueline Goedhals, NHLS and University of the Free State.**

Primary effusion lymphoma (PEL), which accounts for approximately 4% (0.3% in a recent South African article) of all AIDS-related lymphomas, was first described in 1995 by Caesarman et al and was included in the WHO classification of neoplastic diseases of the haematopoietic and lymphoid tissues in 2001. Subsequently, solid (extracavitary) HHV-8 positive lymphomas have been described to occur prior to the development of, or following resolution of PEL. These lymphomas demonstrate morphology, immunophenotype and genotype identical to their PEL counterparts and have been called solid (extracavitary) PEL. The solid PELs have also been described to occur without an associated lymphomatous effusion. The immunophenotypic and genotypic characteristics of PEL suggest that it is a lymphoma composed of the malignant counterpart of a B lymphocyte that has reached a mature stage of development, intermediate between an immunoblast and a plasma cell. PEL cells usually exhibit an indeterminate or null-cell immunophenotype, lacking expression of B cell or T cell associated antigens, but expressing plasma cell markers (CD138 and MUM-1). Variable expression for CD30, CD38 and CD71 is also described. Cases of PEL exhibiting a T cell or biphenotypic B and T cell immunophenotype and genotype have been described. This has been termed "lineage infidelity" and is a rare occurrence in mature lymphoid neoplasms in contrast to precursor lymphoid malignancies.

We present a case of an HHV-8 positive large cell lymphoma, presenting as a soft tissue mass in the thigh of a 31 year old HIV positive male patient. The tumour cells have plasmablastic morphology and a CD45 +, CD20 and CD79a -, OCT-2 and MUM-1 +, CD138 - and CD3 and CD5 + immunophenotype. It is our opinion that this represents a solid variant (extracavitary) PEL showing lineage infidelity.

**APP10: Renal manifestations in children co-infected with HIV and disseminated tuberculosis.**

**W Bates, Division of Anatomical Pathology, Department of Pathology, Stellenbosch University, NHLS Tygerberg Hospital.**

**P Nourse, MF Cotten, Department of Paediatrics, Stellenbosch University, Tygerberg Hospital.**

Many children in Cape Town are co-infected with human immunodeficiency virus (HIV) and tuberculosis (TB). Granulomatous TB interstitial nephritis is a recognized entity. Our objective was to establish if TB plays a role in renal disease in HIV-infected children. We identified children co-infected with TB and HIV from our database and reviewed their biopsies and clinical notes. Since 2002, 12 renal biopsies or postmortem examinations were performed on HIV-infected children at our institution. The clinical scenario and renal biopsies in four cases (median age 73 months, range 24-108 months) were consistent with TB involvement. The mean CD4 count and percentage of these four patients were 508 cells/microl and 23%, respectively. All four patients presented with culture-proven disseminated TB (not yet on treatment) and had nephrotic range proteinuria and hypoalbuminemia. Three of these patients had renal impairment. The prominent features of the renal biopsies were a severe interstitial inflammatory infiltrate and mild to moderate mesangial proliferation. An interstitial granuloma was seen in one patient. With treatment for the TB, the proteinuria resolved and renal function improved in all four patients. Based on these results, we conclude that TB contributes to proteinuric renal disease in HIV-infected children and that the renal disease improves following TB treatment.

**APP11: The necessity of a negative control as well as the stringency of the post hybridization wash in the ISH protocol.**

**MB van Heerden, SC Boy, WFP van Heerden, Department of Oral Pathology, University of Pretoria.**

In situ hybridization (ISH) is a powerful molecular tool used to visualize nucleic acids in tissue and cells. Labelled probes anneal to complimentary nucleic acid sequences allowing microscopic and cellular localization of DNA and RNA sequences in a heterogeneous cell population. Several factors influence the specificity and validity of non radioactive ISH. These include fixation of tissue, tissue processing, choice of denaturation method and stringency conditions of probe hybridization and post hybridization washes. It is important that proper controls are performed to show that the labelling is due to hybridization of the target rather than non specific labelling. This study was performed to determine the cause of false positive/non specific staining when HHV8 ISH was performed on a negative control section according to the specification sheet of the manufacturer. Different antigen retrieval techniques, dilution of solutions, incubation times and post hybridization washes guided by the protocol sheet of the manufacturer, were evaluated. The different antigen retrieval techniques, dilution of solutions and incubation times did not eliminate the non specific binding of the probe. It was found that adjusting the time and temperature of the stringency wash specified by the manufacturer removed the non specific binding of the probe to yield absence of staining in the negative control. This study showed the importance of the duration and temperature of the post hybridization washes in the ISH protocol as well as the necessity of a negative control to validate interpretations thereof.

**APP12: Hormonal status and HER-2 expression in invasive breast cancer, 16 month private laboratory experience from Durban.**

**Ashwin Bramdev, Logan Govender, Lancet Laboratories, Durban.**

**INTRODUCTION.** Breast cancer is the most commonly diagnosed cancer in South African women. It is a disease with considerable heterogeneity in its behaviour, and newer predictors of prognosis and response to therapy are required.

**AIM.** To evaluate the expression of estrogen receptor (ER), progesterone receptor (PR) and HER-2 in breast cancer and compare them with other clinico-pathological parameters.

**Materials and Methods.** This is a retrospective study conducted at Lancet Laboratories, Durban over a 16 month period from January 2009 to April 2010. 337 invasive breast cancers were diagnosed over this period and immunohistochemistry was performed for ER, PR and HER-2.

**RESULTS.** The age ranged from 26 to 87 yrs. 64,4% were ER positive, 45,9% PR positive and 14,8% were scored as 3+ HER-2 positive. 20,5% were negative for ER, PR and HER-2. Correlation with age, histological type and grade will be discussed.

**CONCLUSION.** Our private practice experience of ER and PR expression parallels other studies, with 14,8% of our cancers being 3+ positive for HER-2 on immunohistochemistry. 20,5% are triple negative tumours (negative for ER, PR and HER-2).

**APP13: Dysplasia and squamous carcinoma of the conjunctiva in a cohort of young patients: is this an evolving epidemic?**

**Martin John Hale, Division of Anatomical Pathology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand and NHLS.**

**INTRODUCTION.** Dysplasia of the conjunctiva and infiltrating squamous carcinoma are associated with long term exposure to ultraviolet radiation. Typically, these changes are seen in older patients and in particular those who have lived for extended periods of time in tropical regions of the world.

There is anecdotal evidence to suggest that the expected demographic profile of patients with dysplasia and squamous carcinoma of the conjunctiva is changing towards a younger patient.

**METHODS.** The archives of the Division of Anatomical Pathology at Chris Hani Baragwanath were searched for all conjunctival biopsies in 2007 and 2008 showing dysplasia or squamous carcinoma. Patients 35 years or younger were identified and their pathological changes stratified according to severity of dysplasia and invasive carcinoma.

**RESULTS.** A total of 418 patients with conjunctival biopsies were found, 116 being aged 35 years or younger. Of this group, 51 (44%) showed evidence of dysplasia or invasive carcinoma stratified as follows: 2 patients had mild dysplasia, 8 moderate dysplasia, 21 severe dysplasia / carcinoma in situ and 7 invasive squamous carcinoma. A further 13 had dysplasia not otherwise specified. Dysplastic changes appear to be commoner in the female gender, 34 (66%) as compared to the male, 17 (34%).

**CONCLUSION.** There is evidence to support the anecdotal impression that increasing numbers of younger patients are presenting with conjunctival dysplasia and that this is commoner in the female gender. Reasons for this remain unclear at present, but it is possible that HIV infection or co-infection with an as yet unidentified infectious agent may play an aetiological role.

**APP14: Tissue quality in tissue microarray (TMA) biomarker validation for lymphoma classification.**

**Yvonne Perner, Department of Anatomical Pathology, University of the Witwatersrand, NHLS, Sub-Saharan Africa Lymphoma Consortium (SSALC/NCI), South Africa.**

**Wendy Stevens, Department of Molecular Medicine and Haematology, University of the Witwatersrand, NHLS, Sub-Saharan Africa Lymphoma Consortium (SSALC/NCI), South Africa.**

**Sharlene Naidoo, Department of Anatomical Pathology, University of the Witwatersrand, NHLS, Sub-Saharan Africa Lymphoma Consortium (SSALC/NCI), South Africa.**

**Leona W. Ayers, MD, Department of Pathology, The Ohio State University, AIDS and Cancer Specimen Resource (ACSR/NCI), Sub-Saharan Africa Lymphoma Consortium (SSALC/NCI), United States.**

**INTRODUCTION:** Tissue microarrays present a rapid and economical method to validate large numbers of biomarkers in formalin-fixed paraffin-embedded tissues (FFPET). In this study, we collaborated with the Sub-Saharan Africa Lymphoma Consortium (SSALC/NCI) to analyze the feasibility of using TMA technology for biomarker assessment in classifying a large number of lymphomas retrospectively.

**METHOD:** Forty-two lymphoma cases classified at the Charlotte Maxeke Hospital, Johannesburg, SA, were assembled into a TMA. This was done preparatory to evaluation of larger case numbers. Two (1 mm diameter) tissue cores were extracted from each FFPET block using a manual tissue-arraying instrument (Beecher Instruments, Silver Spring, MD) and placed in a recipient paraffin TMA block. Sections of the TMA were cut, mounted on adhesive slides and stained with antibodies to CD20, MUM-1, CD10, CD3, CD5, Bcl-2, Bcl-6, and Ki-67. Stains were evaluated for strength and consistency of antibody reactions, and concordance with expectations from whole tissue sections. TMA construction and staining was at the Pathology Core Laboratories, The Ohio State University Comprehensive Center's Innovation Center, Columbus, OH, USA.

**Results:** All biomarkers were positive within at least some of the tissue cores. The technical quality of CD10 and Bcl-6 were most adversely affected by tissue fixation. Occasional CD20 biomarkers were faint due to necrosis or poor tissue preservation. The morphology of biomarker positive cells in crushed tissues was distorted but evaluable.

**CONCLUSIONS:** Tissues vary in quality for biomarker identification by immunohistochemistry. Fixation, necrosis and crush artifact present the same challenges as those encountered in whole tissue sections. Antibody optimization may be required for tissues from different laboratories. While TMA technology offers a rapid and economical technology for evaluation of biomarkers in large case numbers, preliminary studies should clarify the variables in tissues at individual locations, remembering diagnostic human tissue is not a consistent analyte for study.

**APP15: Molecular profile of KIT and PDGFRa in Gastrointestinal Stromal Tumours (GIST).**

**Chantal Babb, Desmond Schnugh, Pascale Willem, NHLS and Witwatersrand University.**

Gastrointestinal stromal tumour (GIST) is the most common mesenchymal tumour of the gastrointestinal tract. Since the introduction of tyrosine kinase inhibitors, such as Gleevec®, the treatment of these patients has drastically improved. Heterogenic mutations in the KIT and PDGFRa genes are central to GIST pathogenesis with the most common mutations occurring in KIT exon 11. Most of these mutations indicate that the patient will respond well to Gleevec therapy. Another common mutation is a duplication in KIT exon 9, and these patients require a double dose of Gleevec to get a

similar response as patients with a KIT exon 11 mutation. The molecular test has been established at the Somatic Cell Genetics Unit, Department of Haematology and Molecular Medicine, NHLS, providing this key service to treating physicians in South Africa.

A total of 17 GIST tumour biopsies from South African patients have been tested. Mutational screening of KIT exons 9, 11, 13 and 17 and PDGFRa exons 12, 14 and 18 was done by direct sequencing of the PCR products of DNA extracted from formalin fixed paraffin embedded samples. Twelve patients had mutations in KIT exon 11, most affecting codons 557-560 and one patient had a deletion in PDGFRa exon 18. Four patients had no detectable mutations in the exons screened and included a juvenile and a Neurofibromatosis 1 patient, which is in keeping with what is generally observed in these GIST patients. Five polymorphisms were observed, however this was not surprising, as African populations are known to have a higher genetic diversity.

The mutational screening of KIT and PDGFRa genes informs treatment decisions for GIST patients. With this test now available in SA, the management of these patients can drastically improve. The trend of mutations observed in this preliminary study predicts that patients should respond well to Gleevec therapy.

#### **APP16: The value of polymerase chain reaction in the laboratory diagnosis of clinically suspected onychomycosis.**

**JW Schneider, Division of Anatomical Pathology, Department of Pathology, Stellenbosch University, NHLS Tygerberg Hospital.**

**AY Moolla, HF Jordaan, Division of Dermatology, Department of Medicine, Stellenbosch University, Tygerberg Hospital.**

**S Engelbrecht, Division of Medical Virology, Department of Pathology, Stellenbosch University, NHLS Tygerberg Hospital.**

**R Munbodh, Division of Anatomical Pathology, Department of Pathology, Stellenbosch University, NHLS Tygerberg Hospital.**

**E Wasserman, Division of Medical Microbiology, Department of Pathology, Stellenbosch University, NHLS Tygerberg Hospital.**

**BACKGROUND:** The accurate laboratory diagnosis of onychomycosis remains a challenge. The aim of this study was to evaluate direct microscopy (KOH), PATHPAS method and culture of nail clippings as diagnostic techniques, and to assess the value of PCR for the categorization of fungi into either dermatophytes, yeasts or non dermatophyte moulds (NDM) or a combination of these 3 major groups, as this has therapeutic implications.

**DESIGN:** 44 patients with clinically suspicious distal and lateral- and total dystrophic onychomycosis, were enrolled. Nail specimens were submitted for direct microscopy, culture, histology and PCR respectively. All laboratory tests were performed according to established techniques and quality assurance protocols.

**RESULTS:** PCR demonstrated fungi in all cases, followed by KOH microscopy in 23, PATHPAS in 21 and culture in 10 cases. In 16 cases, only PCR demonstrated fungi. Corroboration of positive results occurred between 18 KOH positive cases and the PATHPAS method, while 3 cases were PATHPAS positive, but KOH negative. In 3 cases, only KOH demonstrated fungi. Culture confirmed 7 cases with *Candida* spp, 1 with *Trichophyton mentagrophytes*, 1 with *Trichophyton* species (speciation not possible), and 1 with *Penicillium* sp. PCR established dermatophytes in all cases, *Candida* in 37 cases, NDM in 37 cases and mixed infection in 37 cases, respectively.

**CONCLUSIONS:** PCR is of value in clinical practice to facilitate accurate diagnosis of onychomycosis. Mixed and NDM infections are more common than anticipated previously. The clinical significance of PCR only positive cases remains unclear. Although PCR can identify

pathogenic fungi and thereby aid in the therapeutic approach, its role as a primary diagnostic tool requires further studies.

### **APP17: Pthisis bulbi with funnel-shaped retinal detachment and osseous metaplasia - a cause of intraocular calcification.**

**Kirstin Coetzee, Martin Hale.**

**Division of Anatomical Pathology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand and NHLS.**

We present a case of a two-and-a-half year old male infant who presented with blindness of the right eye. MRI scan showed intraocular calcification. The clinical differential was retinoblastoma or post-inflammatory dystrophic calcification. An exenteration was performed.

On gross examination, the globe was slightly shrunken and squared-off. Cut section showed a tan coloured, cone-shaped structure running between the iris and the optic nerve in an antero-posterior direction. The cut surface was gritty, and the surrounding vitreous had a glistening appearance.

Microscopy showed features of pthisis bulbi, and confirmed a funnel-shaped retinal detachment. There was osseous metaplasia with bone formation and associated adipose and haematopoietic tissue. There was some histological evidence of chronic uveitis. A differential diagnosis of choristoma was entertained.

We present this case because of the characteristic, but initially perplexing histopathological features and explore the morphological differential diagnosis.

### **APP18: Unusual mammary and juxta-mammary spindle cell lesions.**

**S Pather, CI Ray, MJ Hale, S Ngwenya & D Fassom**

**Department of Anatomical Pathology, School of Pathology, University of the Witwatersrand, National Health Laboratory Service, Chris Hani Baragwanath hospital.**

Spindle cell lesions of the breast represent an interesting diagnostic challenge, as several benign and malignant lesions are included in this category.

We present three cases of spindle cell lesions of mammary and juxta-mammary localisation occurring in female patients aged 72, 39 and 33 years treated by mastectomy(1) and wide local excisions(2).

Morphologic features were assessed using H&E stained sections and all cases were stained for cytokeratin (AE1-AE3 / MNF116), CD34, CD31, SMA and S100.

The lesions were diagnosed as dermatofibrosarcoma protuberans (DFSP), malignant peripheral nerve sheath tumour (MPNST) and nodular fasciitis (NF) respectively.

DFSP of the breast is rarely reported and primarily represents a locally aggressive tumour of the deep dermis and subcutaneous tissue. In our case, fibrosarcomatous areas constituted approximately 10% of the tumour signifying a greater risk of local recurrence and metastasis.

The percentage of patients with MPNST associated and unassociated with Neurofibromatosis 1 is roughly equal. In our case, origin from a neurofibroma was evident. There was no history of neurofibromatosis and no significant radiation exposure.

Nodular fasciitis is a rare pseudosarcomatous lesion that may clinically and radiologically mimic carcinoma when it occurs in the region of breast. The possibility of spindle cell carcinoma should be excluded by immunohistochemical staining for keratins.

Appropriate clinical information, careful exclusion of an accompanying epithelial element in combination with judicious use of immunohistochemistry allows for diagnostic accuracy, which facilitates appropriate treatment of spindle cell lesions of breast.

#### **APP19: Lipomatous haemangiopericytoma – presentation of two cases.**

**Gerhard van der Westhuizen, NHLS, Bloemfontein.**

Haemangiopericytoma was first described in 1942 by Stout and Murray as a presumably pericytic soft tissue neoplasm with a characteristic branching vascular pattern. The branching vascular pattern proved to be a common growth pattern in many soft tissue neoplasms and haemangiopericytoma should be considered a growth pattern rather than a distinctive entity. First described by Wagner in 1870 and recognized as a distinctive entity by Klemperer and Rabin in 1931, solitary fibrous tumour has subsequently been described in various extrapleural sites, including visceral organs and soft tissues. The great histological overlap between solitary fibrous tumour and haemangiopericytoma and lack of clear differentiating criteria lead many pathologists to favour the term solitary fibrous tumour above haemangiopericytoma. Initially described by Nielsen et al, lipomatous haemangiopericytoma comprises a spectrum of lesions characterized by a prominent haemangiopericytomatous blood vessel pattern and the presence of a variable amount of adipocytes in the tumour. These lesions share clinical, pathological, immunohistochemical and ultrastructural features of solitary fibrous tumour. Gengler and Guillou proposed that lipomatous haemangiopericytoma be called a fat-forming variant of solitary fibrous tumour.

We present two cases of fat-forming solitary fibrous tumours diagnosed at our institution. The first occurred in a 17 month old boy and involved the entire circumference of the left lower leg. The lesion measured 220x170x50mm. The second occurred in the right thigh of a three year old boy and measured 110x85x75mm. Both showed a spindle cell tumour with a pattern-less pattern of growth. Dense ropey collagen could be identified in between the spindle cells. The first case demonstrated scattered as well as small lobules of mature adipocytes. Scattered mature adipocytes only were identified in between the spindle cells of the second case. A focal haemangiopericytoma-like blood vessel pattern could be identified. No cellular atypia or mitotic activity was present.

#### **APP20: Case Report: Kaposi Sarcoma of the Appendix.**

**Glenda Ruth Wright, Michelle Dubb, Charlotte Ray, Division of Anatomical Pathology, University of the Witwatersrand / National Health Laboratory Services, Johannesburg.**

**Introduction:** Kaposi sarcoma is one of the most common malignancies found in patients with HIV infection. HIV/AIDS associated Kaposi sarcoma involves lymph nodes and the gastrointestinal tract more frequently than other sites. The gastrointestinal involvement is most often asymptomatic. Involvement of the appendix, either primarily or as part of disseminated disease, is rare, with only nine confirmed cases published in pathology literature to date. Herewith is a report of two cases of Kaposi sarcoma presenting with features of appendicitis.

**Methods and Results:** We searched records at Chris Hani Baragwanath Hospital, Soweto, for coded cases of appendectomy specimens, between 2000 and 2010. During this period, two cases of appendiceal Kaposi sarcoma were identified. The first patient was a 24 year old female whose HIV status was unknown at presentation. Macroscopic examination revealed an inflamed, perforated appendix with serosal haemorrhage. Histological examination showed a vasoformative nodular lesion in the wall of the appendix, with features of Kaposi sarcoma. The second case was of a 40 year old male who presented with an acute abdomen. HIV status was not known at the time of

surgery. At laparotomy, a perforated appendix was found. Histological examination revealed transmural involvement of the appendix wall by Kaposi sarcoma with no evidence of acute mucosal inflammation. Immunohistochemical stains for CD31 and HHV8 were positive in the lesion. Follow-up investigations suggested both appendiceal lesions appeared to be the only gastrointestinal tract lesions at presentation.

Conclusion: Gastrointestinal involvement by Kaposi sarcoma is well documented and common in the setting of HIV/AIDS and is usually asymptomatic. Involvement of the appendix is rare and may be a cause of acute appendicitis as an initial presentation.

## **APP21: Neuroendocrine tumours of the appendix at Chris Hani Baragwanath Hospital.**

**Sharol Ngwenya, S. Pather, C. Ray, Division of Anatomical Pathology, University of the Witwatersrand/ National Health Laboratory Service.**

Introduction: Neuroendocrine tumours are the most common tumours of the appendix. Appendiceal carcinoid tumours are well differentiated neuroendocrine tumours accounting for 25.7% of all gastrointestinal carcinoid tumours. Review of the literature reveals an incidence of 0.32 -0.6% of all surgically removed appendices. Various classifications have been proposed for these tumours. The categories include the classic (insular) type and carcinoids with glandular differentiation (adenocarcinoid tumour of tubular type and adenocarcinoid tumour of goblet cell type). WHO classification includes carcinoid (well differentiated endocrine neoplasm EC-cell and L-cell), goblet cell carcinoid, tubular carcinoid and mixed carcinoid-adenocarcinoma and others.

Objective: A retrospective study was performed of cases received at Chris Hani Baragwanath Hospital to determine the incidence of neuroendocrine tumours of the appendix, architectural classification and intra-appendiceal location.

Method: Snomed search from January 2000 to May 2010 revealed 3439 appendicectomy specimens received at the histopathology department during this period.

Results: 4 female patients between the ages of 18-32 years had an incidental finding of carcinoid tumour (WHO classification - well differentiated endocrine neoplasm) accounting for an incidence of 0.12%. All tumours were located at the tip of the appendix and measured less than 2cm in greatest dimension. Microscopic examination revealed three classic (insular) type carcinoid tumours and one tubular type adenocarcinoid. Acute appendicitis was present in 3 (75%) cases. The tubular type adenocarcinoid was associated with mucinous cystadenoma with low grade dysplasia

Conclusion: Carcinoid tumour is the commonest neuroendocrine tumour in the appendix. We report an incidence of 0.12% at our institution, which is lower than that reported in the literature.

## **APP22: Diagnostic accuracy of liquid-based cytology brushing of upper aerodigestive tract mucosal lesions.**

**Amir Afrogheh, Jos Hille, Division of Oral Pathology, University of the Western Cape, NHLS Tygerberg Hospital.**

**Lithhare Majara, Colleen A Wright, Pawel T Schubert, Division of Anatomical Pathology, Department of Pathology, Stellenbosch University, NHLS Tygerberg Hospital.**

BACKGROUND AND AIMS: Brush cytology is a useful, economical and practical tool in the diagnosis of mucosal lesions. As yet, it has not been extensively used in upper aerodigestive tract mucosal lesions with only a few published studies. Adequacies of material & sampling, quality of staining and cytological experience have contributed to decrease interest in the use of cytology in

this area. This study aims to evaluate the diagnostic accuracy of brushing using the Shandon Cytorich Red liquid medium for mucosal lesions of the upper aero-digestive tract.

**MATERIAL AND METHODS:** This is a prospective study of brush cytology, of consecutive patients with intraoral and laryngeal lesions placed in Shandon Cytorich Red liquid-based cytology medium. The cytology was correlated with subsequent biopsy.

**EVALUATION:** To date 34 patients have been sampled. The cytology was reviewed and graded according to a novel grading system and the diagnoses were compared to the histopathological diagnoses.

**RESULTS:** Of the 34 patients sampled, 25 had malignant diagnoses on histology with cytology diagnosing 19 (76%) correctly. In the 4 benign cases, cytology diagnosed all these correctly.

No false positive cases were observed. The sensitivity was 83%, specificity 100% and accuracy was 85%.

**CONCLUSIONS:** Liquid-based cytology appears to be an easy, accurate, and reliable cytologic modality for the diagnoses of brushings of upper aero-digestive tract mucosal lesions

### **APP23: Combining fine-needle aspiration biopsy (FNAB) and high-resolution melt analysis in the diagnosis of mycobacterial lymphadenitis.**

**Colleen A. Wright, Division of Anatomical Pathology, Department of Pathology, Stellenbosch University, NHLS Tygerberg Hospital.**

**Kim G. P. Hoek, NRF Centre of Excellence in Biomedical Tuberculosis Research/MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Stellenbosch University.**

**Ben J. Marais, Department of Pediatrics and Child Health, Stellenbosch University, Tygerberg Hospital.**

**Paul van Helden, Rob M. Warren. NRF Centre of Excellence in Biomedical Tuberculosis Research/MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Stellenbosch University.**

Tuberculous lymphadenitis is the most common cause of extra-pulmonary tuberculosis (TB) in developing countries, while lymphadenitis caused by non-tuberculous mycobacteria (NTM) requires consideration, particularly in immunocompromised patients and children in developed countries. Fine-Needle Aspiration Biopsy (FNAB) has been shown to be a valuable specimen collection technique, but culture confirmation, mycobacterial speciation and drug resistance testing (if indicated) is often unavailable in TB endemic areas and result in unacceptable diagnostic delay. This is particularly of concern in immune compromised patients and in the paediatric population, who are at risk for more rapid progression of disease

We evaluated the diagnostic value of high-resolution DNA melting (HRM) analysis in the diagnosis of mycobacterial lymphadenopathy using FNAB and an inexpensive transport medium. Specimens were collected from patients referred to the FNAB Clinic at Tygerberg Hospital (June 2007-May 2008) with clinical mycobacterial lymphadenitis. Cytology, culture, and HRM were performed on all specimens. The reference standard for disease was defined as positive cytology (morphological evidence plus mycobacterial visualization) and/or a positive culture.

Specimens were collected from 104 patients and mycobacterial disease was confirmed in 54 (51.9%); 52 Mycobacterium tuberculosis, 1 Mycobacterium Bovis BCG and 1 NTM. Cytology was positive in 83.3% (45/54) and culture in 72.2% (39/54) of patients. HRM identified 57.4% (31/54) of cases. By using the defined reference standard, we recorded 94.0% specificity and 51.9% sensitivity (positive predictive value 90.3%) with HRM analysis.

HRM analysis allowed rapid and species specific diagnosis of mycobacterial lymphadenitis in the majority of patients, permitting early institution of appropriate therapy. The low sensitivity, while consistent with the literature, requires further study for optimization of this technique.

**APP24: Clinicopathological features of disseminated fungal infection in skin lesions in immune compromised patients.**

**Marijke Smit, Johann Schneider, Division of Anatomical Pathology, Department of Pathology, Stellenbosch University, NHLS Tygerberg Hospital.**

**H Francois Jordaan, Division of Dermatology, Department of Medicine, Stellenbosch University and Tygerberg Hospital.**

**Susan Engelbrecht, Division of Medical Virology, Department of Pathology, Stellenbosch University and NHLS Tygerberg.**

**Elizabeth Wasserman, Division of Medical Virology, Department of Pathology, Stellenbosch University and NHLS Tygerberg.**

**BACKGROUND:** To determine the clinicopathological spectrum of disseminated cutaneous fungal infection in skin biopsies from immune compromised patients.

**METHODS:** This is a retrospective study of immune compromised patients who presented with clinically suspected disseminated cutaneous fungal infection. Cases were diagnosed by histological identification of fungal elements in sections from routinely processed skin biopsies, using H&E and PAS&D stains. Records were reviewed for results of fungal culture.

**RESULTS:** The cohort included 56 skin biopsies from 31 patients, aged 19 to 47 years and with a male to female ratio of 11:4. All patients were HIV positive, except for 3 patients, 1 with leukaemia and 2 with renal transplants. Ten cases revealed sparse perivascular and interstitial lymphocytes, macrophages and cell debris in the upper dermis. Poorly developed granulomatous inflammation occurred in 24 biopsies. Fungal load was sparse in 32, moderate in 8 and high in 9 biopsies. The remaining 7 biopsies required meticulous microscopic examination to demonstrate very sparse fungal elements. Fungal culture, done in 19 cases, confirmed *Histoplasma capsulatum* in 3 cases and *Blastomyces dermatitidis* in 1 case.

**CONCLUSION:** Distinct histopathological reaction patterns, ranging from subtle perivascular and interstitial to more pronounced nodular, folliculocentric and diffuse distribution of macrophages, lymphocytes and cell debris, offer valuable clues to the diagnosis of disseminated cutaneous fungal infection. Fungal morphology may suggest a specific fungal species, but cannot be used to accurately speciate fungi. Fungal culture has limited diagnostic value due to low sensitivity. Molecular studies using real time PCR may be useful to confirm the presence of and to speciate fungi in biopsies from patients with suspected disseminated cutaneous fungal infection.

**APP25: Evaluating the BD Focalpoint Slide Profiler**

**Greta Neethling, Division of Anatomical Pathology, Department of Pathology, Stellenbosch University and NHLS Tygerberg Hospital**

**Irene le Roux, NIOH, NHLS, RSA.**

**Johan Neethling, CPUT, RSA.**

**Zeenith Adhikarie, NHLS, RSA.**

Towards the end of 2008, a BD FOCALPOINT SLIDE PROFILER (FP) was installed at the NHLS laboratory, Tygerberg Hospital. It is currently used as a primary screener for conventional Pap smears. This study was performed to assess (1) reproducibility of an abnormal smear, (2) sensitivity of smears initially diagnosed as normal and (3) statistical reliability of the quintile ranking of normal versus abnormal smears. We currently do not rely on the quintile ranking, since our screening populations' abnormality rate is very high compared to first world countries. This study was undertaken to test this hypothesis.

(1) Reproducibility was evaluated by reloading a smear with scant atypia, suggestive of LGSIL, into the FP instrument. An impressive reproducibility of 93% was proven by identifying at least one of the abnormal cells 14/15 times.

(2) Sensitivity was tested by selecting 100 cases initially diagnosed as normal. These were screened by the FP and then interpreted and rapid reviewed by a cytotechnologist. Three cases were amended to abnormal (1 LGSIL, 1 ASC-H, 1 ASC-US) after a full rescreen and a rapid review by two independent cytotechnologists, thus showing a sensitivity of 97%.

(3) To evaluate reliability of the allocated quintile ranking, more than 1000 cases were selected from four of the largest printsets. A comparison between the quintile ranking and the final diagnosis was made. It showed an abnormality rate as follows:

Quintile 1=58.94%; Quintile 2=19.23%; Quintile 3=11.65%; Quintile 4=9.35%; Quintile 5=5.13%.

The FP has proven to be an asset in our laboratory. In addition to improving turn-around-time, its' reproducible and sensitive is vital to good quality assurance practice. Although the quintile ranking results were fairly representative, this study has shown not to rely on the quintile ranking when interpreting the FP data. This may give rise to false positive or false negative diagnosis.