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Poster Abstracts

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KEY

Ⓟ = Presenter

PRESENTER'S INDEX

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P1

Does EGFR and ALK Mutation Status Correlate With Tumour Morphology in Non-Small Cell Lung Cancer?

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Analysis of adenocarcinoma and non-small cell lung cancer (NOS) for EGFR mutations now forms part of the Royal College of Pathologists' lung cancer dataset. Identification of patients harbouring these mutations facilitates delivery of targeted therapies with superior efficacy. Testing of these tumours for ALK has also been introduced in our centre. We assessed our compliance with the College guidelines in this area for 2013 and 2014. In those tumours positive for EGFR or ALK mutations, we examined the original sections to assess any correlation between mutation status and morphological subtype.

96 of the 116 appropriate cases (83%) diagnosed histologically in 2013 were sent for EGFR mutation analysis, increasing to 183/190 (96%) in 2014. 20 of the cases over this time (7%) were positive for an EGFR mutation. Of these, 12 showed an acinar growth pattern, 3 were solid, 1 lepidic, 1 papillary and 1 micropapillary. It was not possible to characterise the growth pattern in two of the cases analysed as cell blocks. The most common mutation, a missense mutation at codon 858 of exon 21, was most frequently associated with an acinar growth pattern.

Of the 2013 cases, 22 (19%) were sent for ALK mutation analysis, compared with 152 (80%) in 2014. Both of the two cases with an ALK translocation (2p23 rearrangement) showed an acinar growth pattern.

Our compliance with College guidelines in sending appropriate lung specimens for mutation analysis is improving. The correlations between mutation status and morphological subtype add to, and are in keeping with, the current body of evidence in this area.

P2

Primary Synovial Sarcoma of the Heart – An Interesting Case Report and Review of Literature

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Seventy five percent of primary cardiac tumours are reported to be benign atrial myxomas. The remaining are malignant tumours with most of them being sarcoma, particularly angiosarcoma and malignant fibrous histiocytoma. Synovial sarcoma of the heart is a very rare malignancy accounting for less than 1% of all primary cardiac tumours. Most of them arise from the pericardium and the right side of the heart and is considered to be highly aggressive with reduced survival rates. Diagnosis in these rare locations is also challenging.

We report a 42-year-old gentleman who presented to us with productive cough, chest pain and paroxysmal nocturnal dyspnea. Echocardiography revealed a calcified left atrial mass arising from the posterior leaflet of the mitral valve and radiologically was thought to be a benign atrial myxoma. Excision was planned with histology showing a malignant biphasic spindle cell tumour exhibiting marked cellular atypia and numerous mitoses. On immunohistochemistry, the glandular component expressed diffuse positive staining for Bcl-2 and EMA with focal positive staining for pancytokeratins. The spindle cell component expressed CD99 and EMA and was found to be negative for CD34, S100, desmin, Melan-A and HMB-45. Cytogenetic testing revealed SS18-SSX1/2 gene fusion with SS18 rearrangement confirming the diagnosis of synovial sarcoma in this rare location. A postoperative computed tomography was performed which showed no evidence of metastasis or primary lesions elsewhere. There was excellent postoperative surgical recovery and adjuvant chemotherapy was considered in the multi disciplinary meeting. Primary cardiac synovial sarcoma is an extremely rare malignancy especially when arising from the left atrium posing diagnostic difficulty mimicking atrial myxoma. In contrast to the poor prognosis mentioned in the literatures, there was excellent recovery in this gentleman.

P3

Histological Findings in Swyer-James (MacLeod) Syndrome: Report From Three Adult Cases Managed Surgically With Emphasis on the Vascular Network and Radiological Correlation.

Ⓟ LM Mellerick; J Dodd; D Healy; A Fabre

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Swyer-James-MacLeod syndrome (SJMLS) is a rare lung condition that manifests radiologically as unilateral hemithorax lucency as a result of post-infectious obliterative bronchiolitis, leading to small airways obstruction and secondary emphysema.

The histological features of SJMLS are poorly and infrequently described. We present three cases of the syndrome that underwent lobectomies in our institution from 2013 to 2015, in three women, aged, 33, 46 and 22 years, presenting with recurrent lower respiratory tract infection, shortness of breath and pleuritic chest pain. Two underwent left upper lobectomies, one left lower lobectomy.

The first case demonstrated hyperlucency of the affected lobe with markedly reduced blood vessel attenuation. The radiological findings of the second case were of extensive bronchiectasis, hyperlucency, mucus plugging and hypervascularity. The radiological findings of the third case were of an apical bulla and upper lobe cavitating lesion with lobar hypolucency and hypoperfusion.

The main histological findings were bronchiolar changes with bronchiolectasis, mucus plugging, constrictive / obliterative bronchiolitis and various degree of peribronchiolar inflammation. Emphysema was mild and diagnosed as loss of attachment of alveolar walls. In addition, Case 1 had dystrophic, hypoplastic or absent branches of the pulmonary arteries. Case 2 showed prominent bronchial arterioles and abnormal tortuous dilated pulmonary arteries and veins. Case 3 had established bronchiolar scars in the bronchovascular bundles, pleural arteries showed medial hypertrophy and the interlobular septa contained dilated prominent veins, as well as cystically dilated inflamed peripheral bronchus.

These cases highlight the importance of vascular changes in SJMLS, likely secondary to the bronchiolar inflammation and destruction leading to capillary bed destruction from secondary emphysema and reactive pulmonary and arterial changes.

P4

Endobronchial Ultrasound (EBUS) is a Highly Accurate and Minimally Invasive Procedure for the Investigation of Mediastinal and Hilar Lymphadenopathy, Both in Malignant and Non-Malignant Conditions.

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Mediastinal nodal staging with EBUS is recommended for patients with resectable non-small cell lung cancer and has emerged as a safe tool to establish granulomatous pathology in suspected sarcoidosis.

Methods: We conducted a retrospective analysis of the outcomes of EBUS performed in a large teaching university hospital with a rapid access lung clinic over a 12 month period and correlation with endobronchial and CT guided biopsies, and surgical resections, when available, and compared the adequacy of EBUS when performed with and without rapid on-site evaluation (ROSE).

Results: In 2014, 161 patients underwent EBUS, 100 males (62.1%) and 61 female (37.9%). Mean age 48.98 years [range 23-89]. A total of 222 lymph nodes were sampled (mean 1.37). ROSE was performed in 80.1%, with a mean of 2.6 MGG slides per lymph node (range 1-12). Cell blocks were prepared in 95% of cases. Immunohistochemistry was performed in 85.5% of malignant cases. Overall inadequacy rate was 9.9%; (8.8 % for sarcoidosis, 15% for malignancy, 11% without ROSE, 5.8% with ROSE).

70.5% of EBUS samples for sarcoidosis show granulomas. Malignancy was diagnosed in 34.2% of cases (pulmonary adenocarcinoma 34.5%, squamous cell carcinoma 26.6%, small cell carcinoma 18.1%, non small cell carcinoma NOS, 7.3%, extrapulmonary malignancies 16.4%). 87.5% of cases with prior history of malignancy or a high degree of clinical concern were diagnosed as malignant. Where available, histological diagnosis concordance was seen in 83.3% of malignant cytology. Molecular studies were attempted on 44.8% of non-small cell pulmonary carcinomas (16 cases); one case demonstrated ALK rearrangement.

Conclusion: EBUS —TBNA of mediastinal lymph nodes has a excellent yield for sarcoidosis and by providing adequate tumour volume, allows for precise subtyping with immunohistochemistry and molecular studies.

P5

Audit of Epidermal Growth Factor Receptor (EGFR) Mutation Analysis in Non-Small Cell Lung Cancer (NSCLC) in an Irish Cohort

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Purpose of Study: Mutational analysis of Epidermal Growth Factor Receptor (EGFR) is now standard practise in selected cases of Non-Small Cell Lung Carcinoma [NSCLC]. An audit was performed to determine the number and histological type of NSCLCs tested with analysis of the frequency and trend of EGFR mutations in our cohort over time, with particular emphasis on comparing the adequacy of histology versus cytology specimens for use in testing.
Methods: Retrospective review of all specimens sent for EGFR mutation analysis in our institution from December 2009 to February 2015. Patient demographics, specimen type, adequacy, EGFR mutation status and mutation type were recorded. Two testing laboratories (both off site) were used, one 2009-Aug 2012 and another Sept 2012-2015.
Summary of Results: 351 specimens from 341 patients were analysed incorporating primary and metastatic lesions. In 13 cases an EGFR result was not available. 29 cases (8.3%) exhibited mutations (13 (44.8%) cytology, 14 (48.3%) histology, 2 modality unknown), occurring in 22 (76%) females and 7 (24%) males. All cases were Adenocarcinomas. Exon 21 (L858R) and Exon 19 (del) mutations were the commonest mutations with 3 patients exhibiting 2 separate EGFR mutations. Mutations were identified in 12 primary tumours (41%), 15 metastasis (52%) and 2 cases were unknown (7%). Up to Aug 2012, mutation rate was 6.5% (153 specimens tested) while thereafter mutation rate was 9.6% (187 specimens tested). 19 (5.4%) sample (s) were insufficient/inadequate for EGFR testing (10 cytology, 9 histology; Fischer's exact test, p=0.1525).
Conclusions: The overall EGFR mutation rate is 8.2%. Cytology Samples are an important source of material for mutational analysis with no significant difference between histological and cytological samples in terms of adequacy.

P6

Cryoprobe Bronchoscopic Transbronchial Biopsies in Interstitial Lung Disease: Experience of an Irish Interstitial Lung Disease Centre

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Background and aims: In Interstitial lung disease (ILD), when an aetiological factor appears absent and clinical-radiological correlation is non-contributory, histology is required. The traditional surgical lung biopsy (SLB) is not without risks. Cryotechnically obtained specimens contain more alveolated lung tissue and less crush artefact than conventional transbronchial biopsies and may offer an alternative to SLB in selected cases. We aimed at studying the complications of cryoprobe transbronchial lung biopsy (CPBx) and the quality and pathological characteristics of the tissue obtained. Methods: This is a prospective study of patients who were selected for CPBx including cases of possible/probable idiopathic pulmonary fibrosis (IPF). Complications of the procedure as well as the quality and pathological characteristics of the tissue are studied.
Results: Twenty-seven procedures were performed in 24 patients, 20 of which were radiologically IPF. A total of 77 biopsies were obtained (Average 2.85 biopsies per procedure). Only one was inadequate initially. Fibroblast foci and features consistent with usual interstitial pneumonia (UIP) pattern were present in 18 biopsies from 16 patients (66.7% of total; 80% of suspected IPF cases). Granulomas were identified in 4 patients (16.7%), 3 of which were radiologically suspected IPF (15% of suspected IPF cases). Two patients (8.3%) had organizing pneumonia; both were inconsistent with IPF radiologically. The findings in the remaining 3 patients were nonspecific; two of these were radiologically IPF (10% of IPF cases). Seven patients (25.9% of procedures) developed pneumothorax, only 2 of them (7.4%) required chest tube drainage. Five patients (18.5%) developed bleeding (moderate in 3 (11.1%) and mild in 2 (7.4%)).
Conclusion: CPBx was useful in this cohort at potentially identifying features not typical of IPF and displayed an acceptable complication rate.

P7

The Amount of Autophagy-Related Cardiomyocyte Cell Death is Associated with the Type of Pathogenic Mutation in Genetic Dilated Cardiomyopathy

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Introduction: Genetic dilated cardiomyopathy is a heterogenous group of diseases caused by mutations in various genes. Several types of cardiomyocyte cell death have been implicated in dilated cardiomyopathy: (macro)autophagy-related cell death, apoptosis, necroptosis and oncosis. One plausible mechanism of genetic cardiomyopathy is proteotoxicity of accumulated protein aggregates. We investigated the association of such aggregates as sign of autophagy-related cardiomyocyte cell death with specific pathogenic mutations.
Methods: Hearts from 30 patients with a genetic dilated cardiomyopathy or a combined phenotype of dilated and arrhythmogenic cardiomyopathy were included. Microscopic slices from 8 regions were immunohistochemically stained for P62, a marker for aggregated proteins destined for autophagy.
Results: Sporadic P62 positive cells were seen in control hearts (0.5% of cardiomyocytes, range 0.1-0.8%). Troponin mutations (TNNT2 and TNNI3; 0.7%, range 0.2-1.2%, n=3) showed hardly any increase in P62. Titin (1.6%, range 0.7-2.7% ,n=5) and lamin A/C (1.7%, range 1.1-2.5%, n=5) mutations showed a threefold increase in P62 staining. A tenfold positive staining was found in desmosomal mutations (PKP2 and DSP; 3.8%, range 3.7-4.0%, n=3) and myosin mutations (MYH7 and MYBPC3; 4,6% range 3.3-5.7%, n=3). Phospholamban mutations (8.8%, range 4.1-16%, n=8) and desminopathies (desmin and Alpha-B crystallin; 17% of cardiomyocytes, range 4.0-31%, n=3) showed the highest number of P62 positive cells.
Conclusion: Accumulation of P62 positive protein aggregates is associated with the type of mutation underlying the dilated cardiomyopathy. Titin, lamin A/C and troponin mutations revealed little protein aggregation, whereas desminopathies, phospholamban, desmosomal and myosin mutations show abundant aggregates. This suggests that the type of mutation plays an important role in determining distinct mechanisms of cardiomyocyte cell death.

P8

Major Trauma Centre Status and its Impact on the Department of Cellular and Anatomical Pathology in a large Tertiary Referral Centre

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Background: Major Trauma has been centralised into Major Trauma Centres which act as the focus of Major Trauma Networks. In April 2012, Derriford Hospital in Plymouth, Devon became operational as the regional Major Trauma Centre for the South West Peninsula. As a result, there was potential for an increased number of trauma-related deaths to be referred to the local coroner, as well as surgical specimens, potentially increasing the work load on pathologists. The case mix could include post-operative cases, neurosurgical cases, polytrauma cases and forensic cases.
Methods: On admission, all eligible trauma patients are recorded onto the Trauma Audit & Research Network (TARN) database. The TARN data was retrospectively analysed and cross referenced with the Department of Cellular and Anatomical Pathologies database to determine how many patients had died, how many had post-mortem examinations were performed and how many surgical specimens were sent, on patients from outside the region or transferred from smaller Major Trauma Units.
Results: Over the first two years, there was a small increase in workload from patients who, prior to Trauma Centre status would have gone to other centres.
Conclusions: In recieving patients from elsewhere in the region, there was an increase in workload for both autopsy and non-autopsy work. This excluded some neurosurgical cases, which traditionally would have been referred (as Derriford is the neurosurgical centre). There are several areas for implication including, APT time, mortuary space and non-autopsy surgical work. Although the workload increase is small, at a time when services are being stretched it is important to ensure any increase in work will not be the "straw that broke the camels back" and can be dealt with accordingly.

P9

An Algorithmic Approach to the Postmortem Investigation and Histological Sampling of Potentially Asbestos Related Deaths

Ⓟ TS Bracey

Derriford Hospital, Plymouth, UK

Purpose of Study To attempt to streamline general pathologist's approach to the investigation of potentially asbestos-related deaths

Methods Turnaround times, tissue sampling protocol and frequency with which samples were sent for formal fibre counts was investigated for 100 consecutive coronial autopsies at the author's institution. Colleagues at other institutions were questioned about their own practice when investigating cases of potential asbestosis, lung cancer or mesothelioma.

Results The author found no consensus in opinion on methods of sampling of the lungs in potential asbestosis, lung cancer or mesothelioma.

The most common indication for samples to be sent for asbestos fibre counts was for malignant mesothelioma. Sending tissue for fibre counts led to considerable delays in the authorisation of postmortem reports and to significant cost implications.

The author presents a pragmatic algorithmic guide to approaching potentially asbestos-related deaths with suggestions for sampling the lungs and tumour in all cases. In general terms, malignant mesothelioma previously confirmed premortem with histology and immunohistochemistry should not require extensive postmortem histological sampling. Lung cancer and asbestosis require widespread sampling of lung tissue to determine amphibole count according to Helsinki criteria in the former, and in the latter, assessment of the distribution and degree of fibrosis in addition to fibre count. One or more of these tissue blocks can be sent for formal counts in equivocal cases after following the algorithmic approach.

Conclusions Although predominantly intended as a pragmatic approach to assist the busy practicing autopsy pathologist, the author believes that the algorithm presented will help departments streamline their approach to these cases and help the relative of the deceased gain access to compensation when appropriate in a more timely fashion.

P10

Focal Lymphocytic Inflammatory Infiltrates in the Myocardium of a Child Found at Post Mortem. Challenge of Diagnosis.

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[1]Leicester Royal Infirmary, Leicester, UK; [2]St George's Hospital, London, UK; [3]St George's University, London, UK

Purpose of the Study: This is a case report of a three year old girl who died suddenly at home. An autopsy was performed in order to determine the cause of death.

Method: An autopsy was conducted which showed no gross abnormalities. Microscopy of the main organs and microbiological samples were taken for further assessment.

Results: Histological assessment of the heart showed multiple small foci of lymphocytes around vessels and within the interstitum of the epicardium, myocardium and subendocardium. These lymphoid aggregates consisted of 20-30 lymphocytes up to larger numbers of 100 lymphocytes collectively. Several foci were present within virtually all of the sections taken in both right and left ventricles. There was no evidence of myocyte necrosis. Immunohistochemistry confirmed they were of T lymphocyte cell origin admixed with smaller numbers of macrophages.

Histology from the respiratory system showed a diffuse subepithelial lymphocytic infiltrate in the larynx and trachea, and the nasopharyngeal samples detected Coronavirus, Adenovirus and two types of Parainfluenza virus. However, viral polymerase chain reaction (PCR) from the cardiac tissue was negative.

Conclusion: An unequivocal diagnosis of a myocarditis could not be made in this case due to the lack of myocyte necrosis and the absence of viral DNA within the cardiac tissue. Genetic testing was strongly advised as splenic material had been taken at autopsy and following molecular genetic techniques a mutation was detected in the sodium channel indicating an inherited ion channelopathy. Further genetic counselling and testing of the remaining siblings and family members is being performed.

P11

Fatal Haemorrhage From Varicose Veins, A Rare Cause Of Death?

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Varicose veins affect a third of the UK population. Isolated case reports and small series of fatalities resulting from varicose vein haemorrhage appear in the literature infrequently. Some of the earliest reports of fatality we have found appear in British Medical Journal (1958) and the Lancet (1973), more recently they have appeared in journals of forensic pathology. Our purpose is to establish and bring attention to the rarity of fatality resulting from varicose vein haemorrhage and the importance of the scene of death and autopsy findings. A literature review was undertaken, we obtained relevant Office of National Statistics (ONS) mortality data for the years 2011-2013, and reviewed our own post-mortem records for demographic, clinical and scene of death information in cases we have encountered.

Our findings confirm that fatality resulting from varicose veins remains a rare cause of death. Some of these deaths are preventable and in 2013 NICE (National Institute of Health and Care Excellence, UK) issued guidelines in which haemorrhage from varicose veins constitute a vascular emergency. Importantly emphasis on first aid is required, simply elevating the limb stops bleeding and is life saving, whereas direct pressure and tourniquets do not.

Pathologists should be aware of potential findings at autopsy in these cases. In particular, awareness that even obscure minor injury to a varicose vein could have resulted in significant blood loss leading to death. Blood lost at the scene will not be apparent at autopsy, and details of blood loss could be variably recorded on the scene of death information provided, therefore vigilance is required.

P12

Unexpected Opportunistic Infections in an Era of Chronic Immunosuppressive Therapy: A Report of 9 Cases.

Ⓟ AM Doyle; N Nolan; T Crotty; A Fabre

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Histopathologists practice in an era of ever advancing medical treatments for a wide variety of oncological, neurological, haematological and rheumatological diseases. Immune modulating therapies are taking a more prominent place in clinical practice. However, with such great advances in therapy comes great risk, with the potential of life threatening opportunistic infections in our patients.

We present a series of 9 immunosuppressed patients who acquired such infections and in whom the diagnoses were made by histopathological examination. The spectrum of these pathogens ranges from viral (CMV, EBV, Herpes), parasitic (strongyloides) to fungal (P. jirovecii, cryptococcus), and the range of infections is diverse. Our series includes 4 males and 5 females, with an age range of 32 - 72 (mean age = 57 years). Unsuspected infectious diagnoses were made at post mortem in 6 of the 9 cases. Organs affected included lung (n = 5), brain (n = 1) and haematological system (n = 1). In one case both colon and lung were affected (n=1) and in a further case both liver and lung were affected (n=1). Immunohistochemistry and/or histochemistry was invaluable in making the diagnoses and was used in all 9 cases (n=9). Treatments leading to immunosuppression included chemotherapeutic agents, monoclonal antibodies, steroids and methotrexate.

We believe that with the ever increasing use of immunosuppressive therapies (both new and old) for a wider number of disorders, vigilance should be paid to their potential to cause life threatening side effects. Histopathologists play a pivotal role in the recognition of this risk and in the diagnosis of these diseases.

P13

Audit of Hospital-Based Adult Autopsy Practice in a University Hospital from July 2013–2014

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The contribution of hospital-based autopsy practice to improvements in patient care is substantial; however, there remains a void in the processes of audit and raising quality of standards in autopsy services. We aim to assess the current autopsy practice compared to RCPath guidelines and identify areas for achieving a high quality autopsy service. All adult autopsy cases performed at a university hospital mortuary between July 2013 and 2014 were reviewed. A total of 522 adult autopsies were performed by 5 Consultant Histopathologists. Ninety nine percent were Coroners' cases. The median turnaround time was 38.5 days, with a range of 3-123 days, excluding 31 outlier cases (complex time-consuming cases). There was considerable variation in turnaround times in complex cases and between the various reporting pathologists. Eighty five percent of cases were compliant with RCPath Minimum Dataset for Autopsy Practice. The remainder were lacking clinical information only. Histology and toxicology contributed to cause of death in 34.4% and 14.8% respectively. No organs were retained. Further review of the cases not compliant with RCPath guidelines (15%), identified that the possible reasons were the inaccuracy, and sometimes irrelevance to the cause of death, of the information received by the pathologists. In many instances, the clinical information given to the pathologist may be controversial, and a certain degree of caution needs to be implemented to avoid including misleading information in the autopsy report. The turnaround times could be improved if a preliminary report is issued within a set time frame, to be followed by the complete report when the histology and toxicology results are available. However, this practice is not acceptable to some coroners who prefer one complete final report. Variations in autopsy practice are to be expected as each autopsy involves substantial case-specific information to which a case-specific answer to the cause of death is expected.

P14

This abstract has been withdrawn

P15

Altered Endosome Biogenesis in Prostate Cancer Identifies Potential Diagnostic Biomarkers

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Prostate cancer is the second most common form of cancer in males, and the incidence of this disease is predicted to double globally by 2030. More than 1.1 million new cases of prostate cancer are diagnosed each year and two thirds of these patients are from the Western world. The current PSA-based test for the diagnosis of prostate cancer lacks specificity, results in missed-diagnoses, over-diagnosis and unnecessary biopsies/treatment. There is an urgent need for a method that enables early accurate detection of prostate cancer.

Endosomes and lysosomes are cellular compartments that degrade and turnover macromolecules in order to maintain cellular homeostasis. These organelles are directly involved in the critical processes of energy metabolism, cell division, and intracellular signalling, which are all hallmarks of cancer pathogenesis. Endosomes have a critical role in controlling the secretion of proteins into extracellular fluids, making them an ideal system to identify new biomarkers that are released from cancer cells. We have discovered that endosome biogenesis (formation and function of endosomes) is altered in prostate cancer. There were significant changes in the gene and protein expression for 19 endosomal proteins and differential distribution of endosome subsets in prostate cancer cell lines. There were also changes to the endosomal traffic and signalling of the transferrin receptor in prostate cancer cells. These fundamental changes in the cell biology of prostate cancer have allowed us to identify a specific set of endosomal proteins that have diagnostic potential. We are developing ELISA's to quantify these endosomal proteins in patient samples and antibodies for immune histology applications. The objective for this project is to develop an effective method for the early and specific diagnosis of prostate cancer, which is important as this will have a major impact on patient outcome and survival.

P16

Langerhans Cell Histocytosis: A Case Report of CT Guided FNAB and Immunocytochemistry

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Objective: Langerhans cell histiocytosis (LCH) LCH is an encompassing term for a spectrum of clinical syndromes characterized by a proliferation of Langerhans histiocytes with other inflammatory cells. LCH is deceiving disease that may be indistinguishable from inflammatory disorders. We reported a case report of Langerhans cell histiocytosis (LCH) diagnosed by fine needle aspiration cytology of bone (FNAB) and immunocytochemistry (ICC).

Methods of Case Report: Six years Egyptian female presented with osteolytic infiltrative lesion at the left head of femur and ischial bone presented for CT guided FNAB.

Results: PAP stained slides and cell block show moderately cellular smears composed of atypical histiocytic cells with abundant eosinophilic cytoplasm and coffee bean shaped nuclei masked by numerous macrophages and eosinophils. The exact morphologic features were confirmed by immunocytochemical (ICC) stains; atypical histiocytic cells displayed positivity for both S100 and CD1a and negativity for CD68. While the macrophages showed positivity for CD68.

Conclusions: CT guided FNAB is very effective tool for rapid accurate diagnosis. Langerhans cell histiocytosis cytologically may be indistinguishable from inflammatory processes therefore cell block for FNAB has a great help for ICC stains confirmation.

P17

MicroRNA Based Molecular Test for Differential Diagnosis of Morphologically Challenging Melanocytic Lesions

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The incidence of malignant melanoma has rapidly increased in recent times and melanoma currently represents the second most common cancer diagnosed in young adults. Diagnosis is based predominantly on histological assessment; however, due to the wide spectrum of morphological characteristics and lack of firm diagnostic criteria, accurate diagnosis can be challenging. Some atypical melanocytic lesions do not display clear-cut morphological features to allow distinction of benign from malignant tumours, making diagnosis and treatment difficult. Among these atypical melanocytic lesions blue nevi, Spitz nevi and dysplastic lesions are common. From histological features alone, it can be difficult to exclude a diagnosis of melanoma and therefore aggressive surgical strategies may be employed in cases were they are unnecessary, highlighting the need for improved diagnostic techniques.
Both mRNA and miRNA profiling have been shown to be able to distinguish benign nevi and primary melanoma tumours. Studying miRNA expression levels is an attractive strategy as miRNAs are highly resistant to degradation and can be easily analysed in FFPE samples. We have studied miRNA expression levels in a cohort of benign, blue, Spitz and dysplastic nevi versus primary melanoma tumours and their derived metastases. Expression levels of key melanoma miRNAs, including miRNA 21, miRNA 211, miRNA 205 and miRNA 200c can be used to distinguish between nevi and malignant melanomas. We propose an easy to implement, simple and robust molecular method based on miRNA expression ratio that, in combination with histological assessment, allows diagnosis of difficult to classify atypical melanocytic lesions.

P18

Effective Molecular Screening in Colorecatl Cancer Patients to Identify Families with HNPCC/Lynch Syndrome in South-East Scotland

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Background: Diagnosis of Lynch syndrome (LS) traditionally relies on clinical criteria to guide diagnostic genetic testing. MMR status of the patient's tumour can help detect Lynch syndrome families as well as having other recognised applications for the patient's management including prognostic and predictive significance. As such, the 'Dataset for colorectal cancer histopathology report' recommendations from the Royal college of pathologists were updated in July 2014 to include screening of colorectal cancer patients under the age of 50 and molecular testing for abnormalities in the mismatch repair genes. In South-East of Scotland we introduced molecular testing to identify individuals at risk of LS. To widen our screening in line with revised guidelines set by European experts, we expanded our cohort criteria to include those between the age of 50 and 60.
Methods: Molecular analysis was carried out on 553 individuals: 446 via 'reflex testing' (newly diagnosed colorectal carcinoma ≤60 yrs, or clinical/ pathological features associated with MMR defects, such as pre-menopausal endometrial carcinoma, multiple tumours and medullary-type carcinomas) and 107 via 'request testing' (clinical criteria and referral dependent). 'Molecular-positive' profiles for LS were identified for genetic pre-testing counselling/diagnostic testing.
Results: 41 patients with potential LS were identified, 24 (58.5%) underwent genetic counselling/testing and 13 cases were confirmed LS with germline pathogenic mutations in the MMR genes. Eight of these were identified using reflex testing.
Conclusion: This is the first UK study to show that screening for LS in patients with colorectal cancer under the age of 60 is effective at identifying families with LS. The testing protocol is in line with the recent recommendations.

P19

Characterising the Oral and Bowel Microbiome as a Prelude to Understanding its Role in Cancer Development Using a Next Generation Sequencing Approach.

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Purpose of Study: The human microbiome is rich and diverse, especially in the oral cavity and gastro-intestinal tract, where it has been shown to be more stable in adults, although various factors such as diet and antibiotics mays influence its composition. This pilot study aimed to examine and compare the oral and gut microbial composition in four individuals using a culture-independent approach.
Methods: Saliva and faecal samples were collected from volunteers within the same day on two separate occasions. The V4 region of the 16S rRNA gene was amplified in all samples and PCR products sequenced on an Illumina MiSeq. Unique barcodes were used to sequence 24 multiplexed libraries together. The data were analysed using the Quantitative Insights into Microbial Ecology (QIIME) software. A second series of 42 samples of faeces from 3 individuals were run to investigate consistency over time.
Summary of Results: Operational taxonomic units (OTUs) were assessed and showed 5 major phyla represented in the saliva samples: Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria and Actinobacteria. Similar phyla except forFusobacteria, were found in the stool samples. The weighted Unifrac PCoA analysis displayed a clear separation of the 2 sample groups, and also showed a more disperse bacterial profile for the saliva samples, based on population sizes, whereas rarefaction curves and unweighted analysis indicated higher bacterial diversity in the stool samples. Each individual could be distinguished either by oral or faecal microbiome. One volunteer who had had previous radiotherapy to the mouth displayed a particularly distinct oral microbiota.
Conclusion: The microbial community profiles of saliva and faecal samples of four individuals were found to be distinct from each other, despite sharing similar phyla. Analysis of multiple samples from each volunteer clearly separated each sample by volunteer and by sample type.

P20

Are Current Automated Approaches for Determining the Phylogeny of Multiple Deposits Capable of Interpreting the Complexity of Cancer Evolution?

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Tumour heterogeneity is central to chemotherapy resistance and disease progression in advanced malignancy. This heterogeneity arises due to the evolution of clones within the tumour cell population; the advent of high throughput sequencing has allowed the detection of different tumour cell clones within and between primary tumours and their metastases, potentially allowing mapping of tumour evolution. Several, automated bioinformatic approaches have been devised for determining tumour phylogeny from changes in genomic copy number (CN); either by the overall similarity of genomic changes between tumour deposits or by examining the occurrence of shared breakpoints.
We have compared these automated approaches with a manual determination of phylogeny based upon shared breakpoints identified from four cases of metastatic colorectal cancer consisting of between 6 and 53 deposits.
We illustrate several recurrent issues identified with the use of automated systems for the determination of tumour phylogeny associated with an inability to correctly identify and interpret changes in ploidy, an inability to identify heterogeneity within tumour deposits, the masking of smaller events by larger ones, over-interpretation of convergent, but unrelated events, over calling sequencing artefacts as changes in CN, and non-calling of genuine CN changes due to low tumour cell content or low sequencing depth.
We conclude that manual interpretation of bioinformatics data is still required to determine the phylogeny of metastatic cancer within an individual.

P21

This abstract is not available before the meeting

P23

Enhanced Regulation of Cell Cycle and Suppression of Osteoblast Differentiation Molecular Signatures by Prostate Cancer Stem-Like Holoclones

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Purpose of the Study: Targeting the stem cell properties of tumor-initiating cells is an avenue through which cancer treatment may be improved. Before this can be achieved, so-called cancer stem cell (CSC) models must be developed and characterized in specific malignancies.

Methods: In this study, holoclone formation assays were used to characterize stem-like molecular signatures for prostate cancer (PCa) cells.

Summary of Results: LNCaP and PC3 parent cells were capable of responding to stem cell differentiation morphogen retinoic acid (RA), suggesting the presence of inherent stem-like properties. LNCaP cells, which represent early, androgen-responsive disease, formed holoclones after twenty six days. PC3 cells, which represent advanced, metastatic, castration-resistant disease, formed holoclones after only six days. Holoclones displayed decreased expression of RA-genes, suggesting a more immature, less differentiated phenotype. Gene and mircoRNA arrays demonstrated that holoclones downregulated a number of stem cell differentiation regulators while displaying enhanced regulation of G2 to M transition and the mitotic spindle checkpoint components of the cell cycle. PC3 holoclones displayed pronounced downregulation of known regulators of osteoblast differentiation from mesenchymal stem cells and Epithelial Mesenchymal Transition.

Conclusion: Our results suggest that some PCa cells retain the ability to transition to a more immature state in which differentiation and metastatic mechanisms are changed. The highlighting of osteoblast differentiation regulators in this mechanism is particularly notable, considering the propensity of PCa to metastasize to bone.

P22

Challenging the Royal College of Pathologists' 2000 Cells Sample Size Required for the Accurate Quantification of Ki-67 Proliferative Indexes (PIs) in Gastroenteropancreatic Neuroendocrine Tumours (GP-NETs)

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Background: The Royal College of Pathologists' guideline on reporting GP-NETs states that Ki-67 PIs should be assessed for grading and suggested a sample size of 2000 cells in areas of highest positive staining. There was little supporting evidence for the suggested sample size.

Aim: To compare the accuracy of counting smaller sample sizes for Ki-67 PIs against 2000 cells as recommend by the Royal College of Pathologists.

Methods: Sections from at least 90 GP-NETs were immunostained for Ki-67 and microscopic images were analysed using ImageJ 'cell counter' tool. Ki-67 PIs were recorded at intervals of 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 cells respectively. The 2000 cell PI was considered the 'gold standard' for comparison. Two independent researchers performed the counting.

Results: Levels of agreement between each sample size and the 'gold standard' were evaluated using Bland—Altman plots. 7 separate pairwise comparisons were performed. Some small sample sizes were shown to have small mean difference and narrow limit of agreement. The Ki-67 PIs were then translated into grades and similar comparisons were performed by calculating the kappa score for categorical variables. Additionally, the interobserver variation between the two independent researchers were calculated.

Conclusion: Smaller sample sizes (below 1000) tend to overestimate the Ki-67 PIs, possibly due to the effect of concentric counting starting from the center of the hotspot. However, the Ki-67 PIs do start to stabilise closer to 2000 (e.g. 1500). The interpretation of whether a lower sample size can replace the current standard would be a subjective decision, but the kappa score gives a rough idea of how much it affects the clinical grading. Updated data will be presented.

P24

Inhibition of MyD88 Facilitates Primed-State Transition During Differentiation of Nullipotent Embryonal Carcinoma Cancer Stem Cells

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Background: Cancer Stem Cells (CSCs) are highly tumourigenic in the undifferentiated state only. As such, forced differentiation of CSCs is a potential clinical CSC-targeting mechanism. Historically, treatment with Retinoic Acid (RA) has yielded strong force-differentiation results in pre-clinical studies but its use does not translate to the clinic. We have previously described a role for Toll-Like Receptor (TLR) Signaling modulator MyD88 in malignancy pluripotency. The aim of this study was to further elucidate this mechanism with a view towards developing improved methods of forced differentiation.

Approach: MyD88 was inhibited (peptide-inhibitor) in nullipotent embryonal carcinoma (EC) CSC cell line 2102Ep [nullipotent] and resulting cell types isolated and analysed via flow cytometry.

Results: Initial studies indicated that inhibition of MyD88 forced 2102Ep CSCs to transition in to a 'Primed Undifferentiated State' (PUS), which was responsive to differentiation by RA. Further analysis demonstrated that 2102Ep cells contain 2 sub-populations, only one of which is capable of RA-induced differentiation via PUS transition. The first population maintains nullipotency through maintained high expression of pluripotency markers SSEA4, Oct4, Sox2 and Nanog, which are lost during differentiation of the PUS sub-population. This mechanism is highly adaptive: PUS CSCs can return to the nullipotent, differentiation-resistant and highly-tumourigenic state if MyD88 inhibition is removed. Similar mechanisms are likely employed in vivo, which may explain the failure of RA treatment strategies in the clinic.

Conclusion: Our data indicates that MyD88 is a Differentiation-Gate-Keeper in pluripotent malignancy. It is likely that specific Gate-Keepers operate in other malignancies. Combining Gate-Keeper targeting with standard CSC morphogens increases force-differentiation efficiency

P25

Platelet Cloaking of Cancer Cells is Universal and Drives EMT

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Background: Systemic spread of primary carcinoma resulting in metastatic disease is the main cause of death from solid tumours, yet the molecular mechanisms driving metastasis are poorly understood. This study focused on the intermediate cells in the metastatic cascade, CTCs. Previously we showed that platelets, through direct interaction, aid survival and drive the metastatic profile of ovarian cancer cells. Here we sought to build on this work by examining if platelet cloaking of cancer cells is universal, whether previously seen epithelial mesenchymal transition (EMT) changes are a constant result and if these changes are associated with alterations in stem
Methods: We examined by flow cytometetry the interaction in vitro between platelets and 15 human cancer cell lines of different origin and metastatic potential. The EMT profile of cells 24hr post platelet exposure was assessed by morphology and gene expression analysis (RT-PCR).
Results: Here we showed that platelet cloaking of cancer cells is universal, occurring across all 7 tumour types examined. However, it is heterogeneous with adhesion rates varying both across and within tumour types, from 35% (PC3-metastatic prostate cancer) to 83% (SKMES1-metastatic lung cancer). Changes indicative of EMT were seen in all cell lines. However, again they were heterogeneous in nature; with morphology changes akin to EMT observed at varying degrees across the cancer types. Also, there was no consistent pattern to the EMT-like gene expression changes seen, with one exception a significant increase in the expression of plasminogen activator inhibitor 1 (PAI-1)was observed in 93% of the cell lines examined.
Conclusion: In this study we describe the universal nature of platelet cloaking and that even though the interaction is not inducing precisely the same molecular changes in all the cancer cells; overall it is driving these cells into a mesenchymal phenotype.

P26

Comparative Assessment of HPV Detection Assays in the Management of Women Referred to Colposcopy with Minor Abnormalities

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Background: Cytology based colposcopy referrals include a substantial number of women with minor abnormalities, only a small proportion of which are at risk of developing high-grade disease. Appropriate management strategies should therefore be investigated. In this study, we evaluated the role of HPV DNA testing and mRNA testing in the management of women presenting in colposcopy with minor abnormalities.
Methods: Study participants were recruited at the Colposcopy clinic in the Coombe Womens & Infants University Hospital following an abnormal cytology referral. At the clinic, a smear sample was taken for cytological evaluation and the residual sample was processed for HPV testing with the Cobas HPV DNA test and the Aptima HPV mRNA assay. Clinical performance of the assays was evaluated in comparison with histological diagnosis.
Results: Clinical performance of the Cobas HPV test and the Aptima assay was evaluated in women referred with minor abnormalities (n=281). Clinical sensitivity and specificity for detection of CIN2+ was 87.8 % and 56.0% for the Cobas test and 87.8 % and 60.4% for the Aptima. Analysis was also stratified by referral cytology and the sensitivity and specificity of the Cobas test in the LSIL category was 88.1% and 48.5% versus 91.5% and 51.4% for the Aptima assay. In the ASCUS category, the sensitivity and specificity of the Cobas test was 87.5% and 65.8%. The Aptima test displayed slightly lower sensitivity at 82.5% but much higher specificity at 72.1%.
Conclusion: Both tests performed comparably, however the Aptima test has better specificity in correctly identifying women at low risk in the ASCUS referral cytology.

P27

Evaluation of Triage Strategies for Management of HPV DNA Positive Women Presenting at Colposcopy

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Background: HPV DNA testing has improved the management of cervical disease. However due to the high prevalence of transient HPV infections, specificity of HPV testing is limited. There is a clear need to improve the specificity of HPV DNA testing in order to avoid un-necessary testing and follow up. In this study we evaluated alternative approaches; HPV 16/18 genotyping, HPV mRNA and p16/Ki67 expression to triage HPV DNA positive cases presenting in colposcopy.
Methods: Patients were recruited at the Colposcopy clinic in the Coombe Womens & Infants University Hospital. A smear sample was taken for cytological evaluation and the residual sample was tested with the Cobas HPV DNA test, Aptima mRNA test and CINtec (p16/Ki67).Clinical performance of the assays was evaluated in comparison with histological diagnosis.
Results: 875 women were tested in total. 57% (310/542) of women with minor abnormalities were HPV positive of which 40.3% had HPV 16/18 genotype and 88% were mRNA positive. 85.2% (284/333) of women with high grade disease were HPV positive of which 60.5 % had HPV 16/18 genotype and 96.1% were mRNA positive. The Aptima test had higher sensitivity to predict CIN2+ at 97.1% compared to HPV16/18 genotyping at 61.7%. The specificity of the Aptima assay was 20% versus HPV16/18 genotyping at 57.2%. 100 patients had all three tests performed. Positivity for Cobas, Aptima and CINtec was 82.3 %, 70.5% and 52.9% in <="" p="">

P28

Development of Diagnostic Test for *H3F3A* p.Gly34Try (G34W) Mutation in Giant Cell Tumours of Bone Based on Droplet Digital PCR

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Giant cell tumours of bone (GCT) are primary locally aggressive bone tumours with a recurrence rate of up to ~30%. The tumour is characterised by numerous osteoclasts and neoplastic stromal cells. Making a diagnosis can be challenging because the differential diagnosis incudes an array of benign osteoclast-rich tumours but also osteoclast-rich osteosarcoma. Recently the occurrence of *H3F3A* p.Gly34Try (G34W) and G34L mutations was reported in 96% of GCT, the latter occurring rarely. These mutations occur in less than 2% of >400 other benign and malignant bone tumours. It has been emphasised that a diagnosis of GCT should be made with caution in the absence of detection of G34W substitution.
Given the diagnostic importance of G34W mutation in GCT, we have developed a simple, quick and cost-efficient diagnostic test to detect this recurrent alteration in FFPE DNA using droplet digital PCR (ddPCR). The ddPCR data from DNA of >100 GCT have been compared with previous 'genotype' data generated using Sanger sequencing, and a number of next generation sequencing approaches (whole exome, whole genome and targeted panels). The G34W and G34L can both be detected in a single assay. We have demonstrated the sensitivity, specificity, repeatability and robustness of the test to be very high with a turnaround time of no more than 5 working days. As ~30% of GCT recur locally following curettage a blood test would be valuable to monitor patients. To this end the ddPCR test also shows that the mutation can be detected in plasma.

P29

IDH1 and IDH2 Digital PCR Assay Development for Chondrosarcoma Tissue and Plasma Analysis

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Conventional chondrosarcoma is the most common bone sarcoma in adults with an incidence of ~0.2/100,000/year. Clinical outcome has not changed over 30 years with only ~50% of patients with high grade disease (Grade (G) II, III) surviving 5 years. Patients with a dedifferentiated central chondrosarcoma, a rare variant, derived from conventional CS have a 5% 5 year survival. Typically resistant to chemotherapy and radiotherapy, high grade disease has been treated by surgery for more than 50 years. It has recently been shown that IDH1 and IDH2 mutations are present ab initio in ~60% of chondrosarcoma cases and that these are retained throughout disease progression. This has opened up a number of new potential diagnostic, biomarker and therapeutic options.

Digital PCR is currently the most sensitive and accurate method for detecting and quantifying mutant DNA molecules. The BioRad QX200 digital PCR platform is also both cost effective and scalable. Using the QX200 platform, we have developed assays for the 5 common IDH1 mutations and the 1 common IDH2 mutation. We have developed the IDH1 assays both in singleplex and multiplex.

We have optimised and validated all assays in tissue samples demonstrating both high sensitivity and specificity when compared to previously genotypes samples. We have demonstrated that the assays are quantitative over 4 orders of magnitude and in high quality DNA we can detect IDH mutations at below 1 mutant molecule in 10,000 wild type molecules.

In a pilot study, we have used digital PCR to analyse circulating tumour DNA levels in plasma taken pre-surgery from 14 patients whose chondrosarcoma harbour an IDH1 mutation. It was possible to detect IDH1 mutant molecules in plasma of all Grade III samples, 50% of Grade II and none of the Grade I samples. In 4 of these cases where the ctDNA was also measured post-operatively, the levels of ctDNA dropped dramatically.

P30

Tumour Necrosis Factor Receptor, CD40, Gene Functions as an Oncogene and Promotes Cell Proliferation in Colorectal Cancer Cell Lines

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Introduction: CD40 is a tumour necrosis factor (TNF) receptor which regulates a range of cellular responses. CD40 is activated by its ligand CD40L and may promote tumourigenesis in haematological cancers. However, CD40 functions as a tumour suppressor in solid cancers. CD40 maps to chromosome 20q13, a region which is amplified in 40-50% of colorectal cancer (CRC). The functional activities of CD40 were tested in CRC cell lines for cell proliferation and motility.
Methods: Expression of CD40 was screened in CRC cell lines by western blot. To define the role of CD40 in human CRC, we knocked down CD40 using small interfering RNA (siRNA) and the knockdown was confirmed by qPCR and western blot. The PrestoBlue assay was used to study proliferation in colorectal cell lines, and flow cytometry to study the cell cycle. Transwell migration and wound healing assays were performed to investigate the effect of CD40 on cell motility in CRC.
Result: CD40 was expressed in CRC cell lines HCT116, RKO, DLD1 and HT29, and not expressed in SW480 and SW620 cell lines. Knockdown of CD40 reduced cellular proliferation in HCT116 (p=0.0158) and DLD1 (p=0.0020) cell lines. Knockdown of CD40 showed a higher number of cells in the sub G0 phase (dead cells) in the cell cycle analysis compared to the control. However, knockdown of CD40 in HCT116 did not have an effect on cell motility in both the transwell migration (HCT116= p=0.253) and wound healing assays.
Discussion: CD40 exhibited oncogenic activity in CRC cell lines. CD40 enhanced cell proliferation but not cell motility in CRC cell lines.

P31

CD45 Positive Small Cell Carcinoma and CD45 Negative High Grade B-Cell Non-Hodgkin Lymphoma

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The expression of CD45 (Common Leucocyte Antigen) and cytokeratin is thought to be mutually exclusive with CD45 expression largely restricted to haematological malignancies and cytokeratin expression largely restricted to carcinomas. We report two clinically relevant cases.
The first case is a urinary bladder biopsy showing a high grade malignant tumour with cells that had scanty cytoplasm, hyperchromatic stippled nuclei and high mitotic activity. Nuclear molding was present. The tumour cells showed focal strong positivity for CK7 and diffuse positivity for CD56 and synaptophysin. Focal positivity for CD45 was present and confirmed on repeat staining. The morphology and immunoprofile was consistent with a small cell carcinoma showing aberrant CD45 expression.
The second case is a maxillary tumour biopsy composed of medium/large atypical lymphoid cells with hyperchromatic nuclei, small nucleoli and scanty cytoplasm. Mitoses and apoptotic cells were noted. Immunohistochemistry showed the atypical cells to express CD20, CD79a, Bcl6 and MUM1 but not CD45, CD5, CD10, cyclin D1, CD30, TdT, ALK1, CD2, CD3, neuroendocrine or melanocytic markers. A high Ki67 proliferation fraction was present. The appearances were consistent with a diffuse large B-cell lymphoma.
The above cases highlight the possibility of aberrant expression as well as loss of expression of immunohistochemical markers by neoplastic cells in undifferentiated malignancies. Attention to tumour morphology may provide diagnostic clues. Interpretation of immunohistochemistry in the context of tumour morphology as well as awareness of aberrant expression/loss of expression can help avoid diagnostic error.

P32

Androgen Receptor Expression in Breast Carcinoma of Egyptian Patients, Correlation with Molecular Subtypes and Prognosis

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Purpose of the study: Breast carcinoma (BC) in Egypt constitutes about 18.3% of total malignancies. Andrgen receptor (AR) is a nuclear hormone receptor that plays a role in hormone receptor- dependent tumors initiation and progression. We investigate AR expression in BC cases, and its correlation with molecular subtypes and survival.

Methods: This study was conducted on 81 cases of BC of Egyptian patients for evaluation of immunohistochemical (IHC) expression of AR.
Results: AR was expressed in 21/81(25.9%) of BC cases. Its expression varied significantly within the molecular subtypes (p<0.001). In particular, AR was expressed in 9.1%, 57.1%, 25%, and 20% of luminal-A, luminal-B, HER-2 positive and triple negative cases respectively. AR expression was significantly related to postmenopausal status, lymphovascular invasion, HER-2 positivity, and high Ki67 labeling index. AR expression doesn't seem to have relation to histologic BC types or overall survival.
Conclusions: AR has significant differential relation to different molecular subtypes. It doesn't seem to have prognostic implications, yet its positivity in significant proportion of BC cases may offer a novel therapeutic target especially for BC cases exhibiting therapy resistance. Further studies are warranted to determine whether AR plays a role in breast cancer or are just a surrogate marker.

P33

A Case of Two Distinct Lesions Within a Lymph Node of a Patient with Invasive Breast Carcinoma

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Accurate, timely diagnosis is the ultimate aim in surgical pathology. Numerous histological pitfalls and lesional mimics exist, with the need to maintain an awareness of such entities vital if potentially serious misdiagnoses are to be avoided.
This case report describes two distinct lesions within the same lymph node, both of which are potential mimics of each other.
A 43 year old female presented with a three week history of a left breast lump. She had no known previous breast disease or any associated risk factors. A needle core biopsy of this clinically and radiologically suspicious mass yielded a diagnosis of grade 2 invasive lobular carcinoma. Left axillary sentinel node biopsy was thus undertaken. Two hot and blue sentinel lymph nodes were excised. One was free of neoplasia, the second contained benign naevus cell inclusions within the capsule together with a micrometastasis. Immunocytochemistry confirmed the presence of two distinct cell populations; the benign naevus inclusion cells stained positively for S100 but not for AE1/3, the reverse pattern was observed in the invasive lobular carcinoma cells. Heterotrophic benign inclusions within lymph nodes are an infrequent yet well recognised entity. Ridolfi et al reviewed the lymph nodes from 909 axillary surgery patients and found 0.017% of lymph nodes contained benign naevus cell inclusions. Small benign naevus cells within the capsule of a lymph node can resemble the 'Indian file' pattern of classic invasive lobular carcinoma.
This case is unusual in that both metastatic carcinoma and benign naevus inclusion cells were present within the same lymph node, enabling a clear comparison of the cytomorphology and immunoprofile of these two distinct lesions.
An awareness of benign inclusions within lymph nodes helps to avoid the potential for misdiagnosis. The judicious use of immunocytochemistry can be useful in distinguishing benign inclusions from carcinoma.

P34

Basal Phenotype Breast Cancer — A Random Designation in Current Practise?

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Recently there has been increasing recognition of distinct breast cancer phenotypes. Of these, Basal phenotype breast cancer (BBC) has attracted particular interest since the majority are triple negative (TN); have an aggressive natural history and can be associated with BRCA1 germline mutation. This area is mired in difficulty, as a precise unifying definition of BBC remains elusive.
Several morphological features more prevalent in BBC have been identified. In our practise we noticed variable use of 'basal' in reports. Given this, whilst no specific therapies to BBC currently exist, we felt it necessary to understand how accurate our designations have been and whether this is a worthwhile practise.
Method: The diagnostic database was searched for all malignant TN breast resections or reports containing the word 'basal' within 12-months. TN was defined as Allred score ER 0-2/8, PR 0-2/8 and HER2 0, 1+ or 2+ negative on FISH. For completeness, we considered including all breast cancers, but pragmatically this was not possible. We carried out CK5 and CK14 staining on all cases where not performed.
Results: Of the 618 invasive breast cancers, 69 cases (11%) were identified, of which 88% were TN and 28% were designated BBC in the report. 16% were both TN and BBC. Where a diagnosis of BBC was made, 37% of cases had additional markers requested. Preliminary results showed 94% were CK5+ and CK14+. This is higher than other studies, implying specificity but not sensitivity in suspecting BBC amongst reporting pathologists.
Discussion: A limitation of this review is that it cannot identify the rare non-TN BBC not diagnosed as such. It also represents current practise in a single institute and may not reflect national practise. We identified patchy use of the designation BBC, with overall under-reporting of this subtype. We recommend that if it becomes necessary to distinguish BBC lesions, additional markers studies, such as CK5 and 14 be consistently performed.

P35

Topoisomerase IIalpha Predicts Survival in Breast Cancer Treated with Neoadjuvant Anthracyclin Based Chemotherapy

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Purpose: Neoadjuvant chemotherapy (NACT) is increasingly used for the management of large but operable, inflammatory, and locally advanced breast cancer (LABC). Little is known about predictors of response/survival following NACT. The topoisomerase IIα (TOP2A) gene, a key regulator of DNA repair and modelling, is thought to be target for anthracyclin and other chemotherapeutic agents. The aim of this study is to assess the role of TOP2A as marker for response/resistance to NACT and patient outcome.
Methods: Patients who underwent NACT, predominantly anthracyclin, for primary and operable invasive carcinoma or LABC in the period between 2005 to 20013 at a single large tertiary referral breast unit were identified. Comprehensive data on chemotherapy regimen, surgical treatment, pathological response and survival were collected. Pre-treatment tumour samples were stained for standard predictive and prognostic markers and TOP2A. Results were correlated with pathological response (PR) and patient survival.
Results: 252 patients fulfilled inclusion criteria. Mean age was 48.94ys. Complete PR was achieved in 15.4%. The mean expression level of TOP2A in pre-treatment core biopsies was 75.4%, range 0-95%. There was significantly higher expression in high grade tumours (p=0.04) and positive correlation with ki67 expression (r=0.405, p<0.001). There was no correlation with nodal status, PR or HER2 expression. Cases with high expression (>50%), had significantly worse overall survival (mean 38 vs 52 months, p=0.01). This was also identified in the endocrine non responsive group (ER Allred score≤4), mean 74 vs 33 months, p=0.04. On multivariate analysis, TOP2A was not an independent factor for overall survival.
Conclusions: TOP2A protein is expressed in high grade breast carcinoma with high Ki67 proliferation index. Its expression in pre-treatment biopsies predicted patient outcome in the neoadjuvant setting. This strong adverse effect on survival warrents further prospective investigation as a marker of outcome in NACT patients.

P36

Breast Cancer Sentinel Lymph Node Diagnosis by Intraoperative Touch Imprint Cytology, a Reaudit of Results to Inform a Multi-Disciplinary Team Evaluation of the Need for New Technologies

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Purpose of Study: Sentinel lymph node sampling is performed in early stage breast cancer, as per NICE guidance, and determines whether axillary clearance is indicated. At Northumbria Healthcare NHS Foundation Trust we use intraoperative touch imprint cytology (TIC) and provide this service across three operating sites (a round trip of 85 miles). It is crucial that sentinel node evaluation is specific to avoid patients undergoing unnecessary axillary surgery. The audit purpose is to measure the quality of TIC against local and published outcomes, and inform an evaluation of the current service versus potential new methodologies for sentinel node sampling.
Methods: Cases included were those coded as sentinel lymph node procedures over a twelve month period. The number of patients undergoing TIC was recorded. The results were compared to the subsequent histology. The sensitivity and specificity of TIC were calculated.
Summary of Results: 123 patients underwent TIC. In seven the result was positive for malignancy and they went on to have immediate axillary clearance. All seven cases correlated with histology positive for macrometastases (specificity 100%, in line with our previous audit and with published figures). 113 TIC cases were reported as negative. Seven of these had macrometastases on histology (giving sensitivity of TIC 50%, in line with published data).
Conclusions: TIC is a procedure with high specificity and this is crucial to avoid unnecessary axillary surgery. Sensitivity, at 50%, is comparable with published figures and previous local audit. New technologies such as PCR may increase sensitivity but costs of implementation at multiple geographically dispersed operating theatres are high.

P37

Her-2 Status on Primary Breast Cancer and Concurrent Lymph Node Metastasis

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Background: Breast cancer is a heterogenous group of neoplasms. Over expression of HER2/neu gene (HER-2) is an important parameter both in terms of prognosis as well as prediction of treatment to anti-HER-2 treatment. Conventionally, HER-2 over-expression is tested on the primary breast tumour and concurrent lymph node metastasis is not usually tested. This study was carried to know if HER-2 status of concurrent lymph node metastasis is similar or different to that of the primary tumour.

Design: 100 consecutive cases of primary breast carcinomas with corresponding lymph node metastases of greater than 2mm were identified. These cases were randomised and anonymised. Immunohistochemistry was performed using an Oracle kit® . The slides were individually scored by 2 independent assessors. All disagreed results were discussed. All slides that scored a 2+ had reflex FISH testing.

Results: In our study, the concordance rate was 87%. Of the 9% that resulted in primary tumour negative and lymph node positive, eight of these cases could not be explained. 4% that resulted in primary tumour positive and lymph node negative, two of these had received neo-adjuvant therapy therefore potentially altering the tumour characteristics.

Conclusion: 30 cases (31%) were HER-2 positive, either primary tumour positive, lymph node positive or both. Testing the primary tumour alone would determine the positivity in 21 cases (21%). However, testing the lymph node would help us determine HER-2 positivity in 26 % of the cases, a difference of 5% . Testing Her-2 status on concurrent lymph node metastasis should also be considered in breast cancer cases.

P38

Reverse Phase Protein Array is a Useful High Throughput Technique for Assessment of Multiple Proteins in Breast Cancer

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Purpose of study: Immunohistochemistry is a well-established and reliable method for assessment of protein expression in breast cancer (BC). However, simultaneous assessment of several proteins is laborious and time-consuming. High-throughput proteomic techniques such as RPPA can provide a quantitative objective alternative with no need for virtual assessment of protein expression.

Methods: A panel (n=16) of biomarkers (hormone receptors, ER-related proteins, HER family proteins and genes related to apoptosis, proliferation and epithelial mesenchymal transition proteins) has been assessed in reverse phase protein array (RPPA). RPPA was applied to 6 BC cell lines representing different molecular classes corresponding to those identified in human BC tissue (Sorlie et al. Proc Natl Acad Sci 2001,98:10869-10874).

Summary of Results: RPPA successfully produced quantitative assessment of several proteins. The differential expression of these proteins in the different BC cell lines was highly comparable to those reported in cell lines and BC molecular classes using other well-established techniques.

Conclusions: RPPA is reliable and useful high through proteomic technique for assessment of large panel of proteins in BC.

P39

Successful Alignment of Macrodissected FFPE Tissue to Nottingham Prognostic Index Plus (NPI+) Classes Using RPPA Technology

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Background: The NPI+ is dependent on the determination of breast tumour biological class by assessing the expression levels of 10 biomarkers using immunohistochemistry (IHC). However, it currently relies on semi-quantitative assessment and does not reflect subtle changes in protein expression. Reverse Phase Protein Microarray (RRPA) can accurately quantitate protein expression and substitute the subjectivity of IHC.
Aim: To validate the use of RPPA in improving molecular classification of breast cancer in order to stratify the patients according to the NPI+ decision making tool.

Methodology: Total protein was extracted using commercial Q-proteome Qiagen tissue kit from 25 cases macrodissected FFPE breast tumour tissues. Samples were robotically spotted onto nitrocellulose-coated glass slide (MicroGridII). Antibodies for ER, PgR, CK5/6, CK7/8, EGFR, HER2, HER3, HER4, p53 and Mucin 1 were used to quantify protein expression along with house-keeping proteins which were compared with IHC.

Results: Enrichment of tumour using macrodissection provided successful correlation to IHC for 8/10 NPI+ markers except HER3 and P53. Classification of tumours into NPI+ Biological Classes using RRPA showed identical classification as IHC.

Conclusion: RRPA is a useful technique for quantification of proteins in FFPE breast tissue enriched for tumour cells by macrodissection and is comparable to IHC.

P40

Improvement of Ki67 Staining in Breast Cancer (BC)

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Introduction: Inclusion of Ki67 into pathology practice improves the performance of prognostication and prediction model of BC. However, the use of Ki67 as part of the daily implemented prognostic indices in diagnostic settings is still controversial. Few reports in literature demonstrated cytoplasmic and membranous expression of a nuclear Ki67 protein in BC which may affect the scoring and cut-off point reproducibility.

Aim: In this study we have evaluation the cytoplasmic/membranous expression of Ki67 using different optimization conditions.

Methodology: Full face sections were stained using different secondary detection kits, different antigen retrieval times, different pH, different protein blocking, different primary antibody (MIB-1) concentration and different primary antibody clones. Additionally, manually and full-automated IHC staining system were used.

Results: There were no obvious differences that could be appreciated regarding cytoplasmic/membranous staining when different antigen retrieval times, different pH, different protein blocking or different antibody (MIB-1) concentration were applied. Furthermore, there was no change in the cytoplasmic/membranous staining in different secondary detection kits when MIB-1 clone was used. Additionally, the cytoplasmic and membranous staining occurred when using the MIB-1 clone to stain the same cases in the Ventana autostainer with different condition. However, use of a different clone (30-9) with the Ventana autostainer resulted in strong nuclear staining with obvious negative cytoplasmic and membranous reactivity.

Conclusion: Using (MIB-1) anti-Ki67 clone with different optimization conditions is associated with case-specific cytoplasmic/membranous reactivity. In this context, it could be suggested that to utilize different anti-Ki67 clones in order to reach consistency in scoring and subsequently cut-off point.

P41

Role of Mitotic Checkpoint Proteins (BUB Family) in Breast Cancer

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Background: The budding uninhibited by benzimidazoles (BUB) gene family encodes protein components of the mitotic spindle checkpoint machinery. Mutations in BUBs may disrupt the checkpoint and cause chromosomal instability, a hallmark of solid tumours including breast cancer (BC). BUBs are among serine-threonine kinases known to help identify luminal BCs with poorer prognosis [Finetti et al, Cancer Res 2008; 68(3)].This study investigated the role of this kinase family at the protein level, especially within low grade BCs.

Methods: BC tissue microarrays (n=1117) were immuno-stained for BUBs (BUB1, BUB1B & BUB3) and expression patterns correlated with clinico-pathological/molecular variables as well as patient outcome. Computational platforms explored the role of the proteins further in relation to BC grade.

Results: Cytoplasmic BUB1 and nuclear BUB3 revealed positive correlations with luminal-enriched proteins like ER (p=0.012), PR (p<0.001) whereas cytoplasmic BUB1B was negatively correlated (p=0.002). BUB1 and BUB3 were negatively associated with grade and NPI, whereas BUB1B was positively associated. Negative associations were observed between BUB1, BUB3 and proliferation markers, Ki67, p16 and PI3K (p=0.04). Positive correlations were observed with STAT3 (p=0.003) including ER positive (p=0.010) subgroups. Survival analysis revealed associations between BUB1 and long term (15 years) breast cancer specific survival in the whole series (p=0.025) as well as ER positive (p=0.024) subgroups. Computational analysis confirmed BUB3 as predictive of grade in BC (p=0.025) with no added value from Ki67 inclusion.

Conclusions: BUB1 and BUB3 are the key kinases for low-grade ER positive BCs and probably maintain genomic stability, whereas BUB1B is preferentially expressed in high grade disease. Functional studies will further delineate the role of these molecules in low grade BCs. *Project supported by CDF from PathSoc and NIHR

P42

Nottingham Prognostic Index Plus (NPI+): Validation of the Modern Clinical Decision Making Tool in Breast Cancer in an Independent Series

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The Nottingham Prognostic Index Plus (NPI+) is a modern clinical decision making tool in breast cancer (BC) aiming to provide improved patient outcome stratification superior to the traditional NPI. This study aimed to validate the NPI+ in an independent series of BC. 885 primary early-stage BC cases were immunohistochemically assessed for 10 biomarkers [Cytokeratin (Ck)5/6, Ck18, EGFR, Estrogen Receptor (ER), Progesterone Receptor (PgR), HER2, HER3, HER4, Mucin 1 and p53] and classified into biological classes. Subsequently, NPI+ Prognostic Groups (PGs) were assigned for each class using standard prognostic variables. PGs were compared between the Validation and Development series and their role in patient outcome prediction.

There was a comparable distribution of biological classes between the Validation and Development (n=1,073) and series. PGs were comparable in predicting patient outcome between series in Luminal A, Basal p53 altered, HER2+/ER+ tumours. The good PGs were similarly validated in Luminal B, Basal p53 normal, HER2+/ER- tumours and the poor PG in the Luminal N class. Due to small patient numbers assigned in the remaining PGs, Luminal N, Luminal B, Basal p53 normal and HER2+/ER- classes could not be validated.

This study shows the distribution of the NPI+ Biological Classes is similar in an independent series of primary BC and can conclude that biological class determination using the NPI+ biomarker methodology is similar between patient series.

We observed similar patterns of patient outcome in the majority of NPI+ PGs between the Development and Validation series and can conclude that NPI+ prognostic classification for these groups appears robust. Three of the poor PGs were under-represented in the Validation series due to a lower frequency of higher grade tumours and could not be validated in this study.

P43

MED7 in Breast Cancer: Relationship to Grade and Lymphovascular Invasion

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Hypothesis: Mediator complex (MED) proteins, components of the transcription apparatus, have a key role in the transcriptional regulation of genes involved in cancer, including the regulation of ERα in breast cancer (BC). Thus the role of the member MED7 was deemed of interest, especially in low grade ER positive BCs. Differential expression analysis between BCs with positive versus negative lymphovascular invasion (LVI) status on the Nottingham subset of the METABRIC series showed that MED7 was negatively correlated with LVI (p=0.005). Thus its protein expression was also investigated in relationship to LVI, alongside other prognostic parameters.

Methods: Breast cancer tissue microarrays (n=1260) were immuno-stained for MED7 and expression patterns were correlated with clinico-pathological and molecular variables as well as patient outcome.

Results: Positive nuclear MED7 expression was significantly correlated with positive ER (p=0.001) and PR expression (p<0.001) and was preferentially positive in lobular carcinomas. MED7 expression was associated with tumours of a low grade (p<0.001), small size (p<0.001), good NPI (p<0.001) and negative HER2 expression (p=0.043). A significant negative association was found with LVI (p=0.025) and proliferation markers like Ki67 (p = 0.001). Kaplan Meier survival analysis revealed significant associations with long term breast cancer specific survival (BCSS) even after 15 in the whole BC series as well as ER positive subgroups (p<0.001).

Conclusions: MED7 was significantly associated with low-grade, ER positive tumours and with negative LVI status. This explains its association with increased long-term BCSS and suggests it as a predictor of favourable prognosis.

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P44

Phenotyping Characterisation of Breast Cancers: the Role of CDC Proteins (2, 42 & 42BPB)

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Background: The molecular profile of low grade (LG) luminal breast cancers (BCs) remains unresolved. Usually of good prognosis, a small subset will progress. CDC (cell division control) family genes are among serine-threonine kinases known to help identify luminal BCs with poorer prognosis [Finetti et al, Cancer Res 2008; 68(3)]. In other studies, CDC42 signalling pathways seem to be active in luminal BCs [METABRIC: Curtis et al, Nature. 2012; 486]. This study investigated the CDC kinase family members at the protein level for correlations with morphology, grade, phenotype and prognosis. Method: BCs (n=1048) of a well annotated series of tissue microarrays were immuno-stained for CDC proteins [2, 42 and 42BPB (binding proteinB) and their expression correlated with clinico-pathological parameters and outcome. Computational platforms explored the role of the proteins in relation to BC grade.

Results: CDC2 and CDC42BPB showed positive association with grade (p=0.015) while CDC42 showed negative association (p=0.009). CDC2 and CDC4242BPB showed negative correlation with ER but positive correlations with HER and basal markers (p=0.001). In contrast, CDC42 expression was positively correlated with PR (p=.044) but negatively with HER2 and basal markers (p=0.03). CDC42 was associated with the tubular and lobular morphology within the low grade BCs (p=0.02) and was associated with longer overall survival (p=0.02) in the whole cohort as well as ER+ (p=0.03) subgroups. Computational analysis confirmed CDC2 as predictive of higher grade in BC (p=0.007) with no added value from Ki67 inclusion.

Conclusion: CDC42 is a key kinase for low-grade BC showing correlations with morphology and receptor status, while CDC2 and CDC42BPB are preferentially expressed in higher grades. Associations of CDC42 to BC morphology probably stem from its role in cytoskeletal remodelling.

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P45

The Prognostic Significance of STAT3 Expression in Invasive Breast Cancer: an Immunohistochemical and Reverse Phase Protein Array Study

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Purpose: Signal transducer and activator of transcription-3 (STAT3) is a STAT family member involved in cellular biological functions. However, reports regarding the prognostic impact of STAT3 expression in breast cancer (BC) were variable whether being a factor of poor prognostic or good prognostic impact in BC.

Methods: Immunohistochemical (IHC) expression of phosphor-STAT3 (pSTAT3) was studied in large series of invasive BC (n=1270). pSTAT3 and STAT3 were quantified using Reverse phase protein array (RPPA) on proteins extracted from macro-dissected FFPE tissues representative of 49 cases. STAT3 transcript data from previously generated gene expression data of 128 frozen BC samples were also analysed.

Results: pSTAT3 was expressed in the nuclei and cytoplasm of invasive BC cells. pSTAT3N+ over-expression was positively associated with good prognostic criteria including small tumour size, low grade, good NPI, negative lymphovascular invasion (LVI), positive ER+, PgR+, negative p53, HER2 negative, and low Ki67LI. However, pSTAT3C+ showed significant positive association only with HER2 status. Nucleo-cytoplasmic combinatorial groups were significantly associated with grade, size, NPI, LVI, ER/PgR status, HER2, P53, Ki67LI, and BC molecular classes. Conclusions: Only pSTAT3N+ was significantly associated with improved breast cancer specific survival (BCSS), independently of other factors. pSTAT3 combinatorial phenotypes were significantly associated with BCSS. On RPPA, the mean pSTAT3 and STAT3 expressions were higher in ER+, PgR+, and tumours smaller size. Lower STAT3 gene transcripts were observed in samples from invasive BC cases which had developed distant recurrence. These associations did not reach statistical significance.

The results of this study suggest pSTAT3 nuclear localisation, regardless of the cytoplasmic expression, to be a marker of favourable prognosis. STAT3, therefore, could have context-dependant molecular roles of STAT3 in BC progression.

P46

Heterogeneity of Ki67 Expression in Different Quadrants and Matched Axillary Nodal Metastases of Invasive Breast Cancer

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Purpose of the Study: Cellular proliferation could be reliably assessed through the expression of Ki67. However, intratumoural heterogeneity (ITH) of expression is amongst the several technical issues beyond the lag of its inclusion into the routine BC practice.

Methods: We studied the IHC expression of MIB1 anti-Ki67 antibody in a subset of invasive (n=55) BC using 3-4 full face (FF) tissue sections from different primary tumour quadrants and the matched axillary metastasis in lymph node (LN) positive cases. Assessment was made following the highest expression (HE, hot-spot), lowest expression (LE), and overall/average expression (AE) in each FF section. Co-efficient of variation, Bland-Altman plots of differences, and Spearman rank correlation co-efficient were used to assess the ITH of Ki67 expression within the same section, between different sections, and between the primary tumour and LN metastases.

Results: Ki67 expression within the invasive tumour was highly variable with more variability in cases of mixed histologic types displayed the highest spatial ITH compared to those of pure special types. Variability of Ki67 HS, AS, and LS within the same FF section ranged from 13-96%. Moreover, variation between the HS, AS, and LS in the studied sections was 2-88%, 0-87%, and 0-96%, respectively. Ki67 expression was perfectly correlated (r=0.98, p < 0.001) with the highest Ki67 expression in the studied FF sections from the primary tumour.

Conclusions: The spatial heterogeneity of Ki67 expression in invasive BC was evident within a single as well as multiple FF sections from the primary tumour. Using the HS/hot spot scoring in FF is more representative of the growth fraction rather than the AS recommended by the Ki67 in BC working group. The high Ki67 within LN metastasis corresponded to highest primary tumour expression reflecting the temporal HT through clonal expansion.

P47

The 2013 American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for HER2 Testing in Breast Cancer increases the requirement for Reflex In Situ Hybridisation Testing

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Purpose: Accurate determination of tumour human epidermal growth factor receptor type 2 (HER2) status is critical for optimal treatment of breast cancer. In October 2013, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) issued joint updated guideline recommendations for HER2 testing in breast cancer, with a revised algorithm for interpretation of immunohistochemistry (IHC) and in-situ hybridisation (ISH) results. This study investigates the impact on HER2 IHC categorisation, implication for reflex ISH testing and potential for identification of false negative IHC.

Methods: HER2 IHC preparations on 251 invasive breast tumours, originally reported according to 2007 guidelines, were re-scored using 2013 guidelines and the diagnostic categories compared. The results of ISH testing on a separate cohort of 32 breast tumours reported as Her2 IHC 2+ following the introduction of the 2013 guidelines, that would have been designated 1+ according to 2007, were reviewed.

Results: Application of 2013 guidelines resulted in a decrease in tumours classified as HER2 negative (83/251 vs 144/251) and a comparable increase in those classified as equivocal (2+) (139/251 vs 80/251). Relatively few tumours were re-classified as positive (29/251 vs 27/251). 3/ 32 breast cancer cases (HER2 IHC 2+ as per 2013 guidelines, 1+ using 2007 guidelines) were HER2 ISH positive.

Conclusion: Application of the 2013 guidelines increases the HER2 IHC equivocal (2+) category and requirement for reflex ISH testing. The reduced threshold for ISH testing identifies some patients with HER2 positive breast cancer whose tumours would have been categorised as HER2 negative according to the 2007 guidelines.

P48

Pathologic Response to Neoadjuvant Treatment and Biomarker Profile in a Breast Cancer Cohort

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Purpose of Study: Extent of tumour response to neoadjuvant therapy for breast cancer is variable and correlates with patient and tumour characteristics.

The aim of this study was to evaluate pathologic response to neoadjuvant treatment and correlate response to tumour biomarker profile in a breast cancer cohort.

Methods: Sequential neoadjuvant treated breast cancer cases were identified over the study period. Biomarker profile was evaluated by IHC (ER, PgR, HER2) and Brightfield HER2 DNA in situ hybridisation (Ventana Inform).

Pathologic response to treatment was evaluated and residual cancer burden (RCB) score and class (complete pathologic response (pCR), RCB1, RCB2 and RCB3) generated using an online tool (MD Anderson Cancer Centre).

Summary of Results: 123 patients with available biopsy and excision data were identified. Biomarker profiles (pre-treatment core biopsy testing) were as follows: Hormone positive, HER2 negative: 52.8%; Hormone positive, HER2 positive: 19.5%; Hormone negative, HER2 positive: 14.6%; Triple negative: 13%. Cases were categorised as pCR (14.6%), RCB1 (6.9%), RCB2 (36.9%) and RCB3 (41.5%). Results for combined pCR and RCB1 groups were: Hormone positive, HER2 negative: 10.8%; Hormone positive, HER2 positive: 33.3%; Hormone negative, HER2 positive: 44.4%; Triple negative: 18.8%.

Conclusions: Hormone positive, HER2 negative tumours were the largest group to receive neoadjuvant therapy in this study, however showed a low frequency of substantial pathologic treatment response. HER2 positivity correlated with highest frequency of complete or near complete pathologic response.

P49

Clinico-Pathologic Characteristics in Neoadjuvant Breast Cancers: A Single Institution's Experience

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Aim: Increasing numbers of breast cancer patients are being treated with neoadjuvant therapy. Pathologic evaluation of post neoadjuvant surgical specimens offers a unique opportunity to assess tumour response to systemic agents and allows evaluation of post-treatment prognostic parameters. The aim of this study was to evaluate the clinico-pathologic features in a large cohort of neoadjuvant treated breast cancers.
Methods: All patients receiving neoadjuvant therapy followed by primary breast excision were identified from institutional files over a 62 month period. All specimens were processed in a single laboratory with uniform standardized pre-analytics, macroscopic and microscopic evaluation and calculations performed as per Residual Cancer Burden (RCB, MD Anderson Cancer Centre Calculator).
Results: 130 patients were identified who had received neoadjuvant therapy followed by breast excision over a 62 month period. Mean age: 51.7 years. 40% had wide local excision, 60% had mastectomy. Core needle biopsy histologic subtype; ducal carcinoma 82.5%, lobular carcinoma 9.7%, metaplastic carcinoma 2.9%, mixed ductal and lobular carcinoma 2.9%, mucinous carcinoma 1.9%. Core needle biopsy tumour grade; grade 3 60.8%. Tumour grade changed between biopsy and excision in 27%, downgraded in the majority (77.8%). Pre neoadjuvant hormone and HER2 status; 52.9% hormone positive/HER2 negative, 19.5% hormone positive/HER2 positive, 14.6% hormone negative/HER2 positive, 13% triple negative. Hormone and/or HER2 status changed in 33.7% post-neoadjuvant cases. Of these, PR changed in 25.3%, ER changed in 9.6% & HER2 changed in 7.2%. The RCB classes were calculated as complete pathologic response (pCR), RCB1, RCB2 & RCB3 in 14.6%, 6.9%, 36.9% & 41.5%, respectively.
Conclusions: In our institution the majority of patients chosen for neoadjuvant therapy are Grade 3, ER positive/HER2 negative tumours. Tumour grade changed in 27%, hormones and/or HER2 changed in 33.7% and 14.6% had pCR.

P50

Invasive Breast Cancer in Women over 75: An Evaluation of Predictive Markers and Aggressive Subtypes

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Purpose of the Study: Breast cancer is a common disease of the elderly with incidence increasing with age. Many studies site increased comorbidities, higher stage at diagnosis and less effective treatment as the reasons for poorer survival rates.
The aim of our study was to evaluate poor prognostic parameters including histologic grade and predictive markers in elderly women with invasive breast cancer.
Methods: All patients aged 75 years and over at the time of diagnostic breast core needle biopsy were identified from the institutional files of a tertiary referral subspecialised breast service over a 5year period (2010-2014). All core needle biopsies were taken from the primary breast cancer and processed in a single laboratory with standardised uniform pre-analytics. Hormone and HER2 evaluation with IHC and Brightfield dual in situ studies were performed as per UK recommendations.
Results: 1603 patients were diagnosed with breast cancer during this time period. 374/1603 (23.3%) were 75 years or over at the time of diagnosis. 348/374 (93%) had invasive carcinoma. Age range for these 348 patients was 75-99 years with a mean age of 81.9 years. Histologic subtypes; 76.2% ductal carcinoma, 18.7% lobular carcinoma, 2.3% mutinous carcinoma, 1.1% papillary carcinoma, 0.9% mixed ductal and lobular carcinoma, 0.4% micro papillary carcinoma and 0.4% mixed tubular and cribriform carcinoma. Histologic grades; 30.7% grade 3, 58% grade 2, 11.3 % grade 1. HER2 profile; 86.3% HER2 negative, 13.6% HER2 positive. Triple negative tumours; 9.2%. In total, 36.8% tumours were either grade 3, HER2 positive or triple negative.
Conclusion: Our study showed that over one third (36.8%) of women with breast cancer diagnosed over the age of 75 had poor prognostic parameters including grade 3, HER2 positive or triple negative tumors.

P51

Mitotic Activity in Benign and Malignant Fibroepithelial Lesions

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Introduction: Classification of fibroepithelial lesions into fibroadenoma, cellular fibroadenoma and phyllodes tumour is challenging and based on features including stromal cellularity, stromal mitotic activity, stromal overgrowth and cytological atypia. Brisk mitotic activity is associated with a diagnosis of phyllodes tumour. The proportion of benign otherwise typical fibroadenomas bearing low level mitotic activity is not well documented.
Aim: To document mitotic activity in a series of fibroepithelial lesions.
Methods: The laboratory information system database was searched for cases coded as 'Fibroadenoma' and 'Phyllodes tumour' from 2008 to 2013. Retrieved case reports were analysed for diagnosis, mitotic activity, tumour border, stromal cellularity, stromal overgrowth and the presence of malignant heterologous features. The data was compiled and analysed with Microsoft Excel.
Results: 1233 fibroepithelial lesions were retrieved and analysed and 64 mitotically active lesions were identified (5.2%). Of these, 50% were fibroadenoma (FA), 22% Benign Phyllodes Tumour (PT), 16% Borderline PT & 12% Malignant PT. Mitotic activity was higher in PT than FA (56% vs. 0.07 % > 4/10HPF) but FA did have mitotic activity (0.39% >2/10HPF). Average age at diagnosis was higher for malignant PT than FA (49 vs 39 yrs). Tumour borders were well circumscribed for FA and benign PT (66 and 86%) in contrast to infiltrative foci in borderline and malignant PT (75 and 71%). While stromal cellularity was noted to be increased in most lesions, stromal overgrowth was more likely to occur in malignant and borderline PT (100%) than in FA and benign PT(38 and 55%).
Conclusion: Occasional mitotic figures may be identified in benign fibroepithelial tumours, including fibroadenomas. Mitotic activity should be considered with other parameters to minimise the risk of misclassification.

P52

An Audit of Breast Cancer ER Positivity Reporting Rate in Wales

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Background: Immunohistochemical oestrogen receptor status (ER) is an important index of tumour prognosis and treatment selection for breast cancer patients. Inaccurate ER analysis has serious clinical implications particularly since a false-negative result leads to patient not receiving the highly effective anti-oestrogen treatment. Following concerns reported in 2012 regarding under reporting of ER status at a UK centre, an audit of Wales laboratories routinely testing ER was conducted. Aims and Objectives: To determine ER positivity rates in Welsh National Health Service (NHS) laboratories to identify any under reporting.
Methods: ER results reported at 5 Welsh NHS laboratories (A-E) in 2012 were retrospectively assessed and sub-divided into screen detected (SD), symptomatic (S) or recurrent/metastatic (RM).
Results: The numbers and respective ER positivity rates (%) of SD, S & RM cases for each centre were determined to as follows: A: 210 (97.1); 235 (89.4); 0 (N/A); B: 94 (89.3); 212 (77.8); 2 (50); C: 178 (93.8); 245 (88.5); 0 (N/A); D: 64 (96.9); 494 (92.9); 16 (50); E: 0 (N/A); 91 (81.6); 7 (57.1). The average ER positivity rate of 89.8% for primary tumours is comparable but higher than UK-wide average rate of 83.7% (UKNEQAS audit). The reason for this higher rate is not certain but may relate to methodological or demographic differences.
Conclusions: 1) There is no evidence of under reporting of ER in Wales; 2) screen detected cancers have a higher ER positivity rate compared to symptomatic and recurrent/metastatic cancers as expected.

P53

Audit of Fibroepithelial Tumours in a Nigerian Tertiary Institution

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Fibroepithelial lesions are the commonest lesions of the breast seen in our laboratory consisting of fibroadenomas and Phyllodes tumours (PT). PT is a rare fibroepithelial breast neoplasm accounting for 0.3-0.9% of all primary breast tumours. The aim of the study was to audit all fibroepithelial lesions and to reclassify all confirmed cases of Phyllodes tumour seen in the study period according to the criteria proposed by World Health Organisation (2003).

Records and slides of fibroepithelial lesions of the breast received at the department between January 2008 and December 2013 were retrieved and reviewed by the authors.

Out of the 1242 fibroepithelial lesions of the breast retrieved, all but 19 were fibroadenomas. The 19 were initially reported as PT: 11 benign, 2 borderline, 2 malignant and 4 unclassified; however only 16 of these 19 PTs (84%) met the WHO criteria on review. The remaining 3 (16%) turned out to be fibroadenomas based on the absence of stroma overgrowth and hypercellularity. The PTs were reclassified into benign PT, borderline PT, and malignant PT accounting for 75% (12/16), 18.7% (3/16) and 6.3% (1/16) respectively. All of the PTs previously unclassified turned out to be benign PTs. One of the borderline PTs was originally reported as malignant PT. All cases initially diagnosed as fibroadenomas did not change on review.

These results show that fibroadenomas are rarely misdiagnosed. The 3 cases misdiagnosed as phyllodes may have been prevented if standard data sets were in use. Cases simply referred to as PT without further classification, limit the patients' access to appropriate management as accurate classification helps in the overall management and prognostication.

P54

A Review of Columnar Cell Lesions in Fibrocystic Changes of the Breast in a Tertiary Centre; A 10-Year Retrospective Study

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Columnar cell lesions (CCLs) of the breast are now gaining clinical attention because of the increasing use of mammographic screening as one of the breast cancer prevention strategies. CCLs are important because certain variants may be precursors of low-grade ductal carcinoma in situ (DCIS). In Nigeria and much of the developing world, mammography is novel and CCLs have not been studied among the black populations as a result.

The aim of this study was to estimate the occurrence of CCLs in Nigerian women using fibrocystic changes as a surrogate lesion as well as determining the range of CCLs found using standard histologic criteria. The slides and records of all consecutive breast biopsies that were diagnosed with fibrocystic changes (FCC) within a 10-year period at the department were reviewed by the authors.

Thirty cases of CCLs were identified among the 559 cases of FCC found. The ages of the patients ranged from 16years to 63years, the age group with the highest incidence was in the 5th decade accounting for 26.7% while the least incidence is in the 2nd decade of life accounting for 9.8%. The commonest pattern found was Columnar Cell Change (CCC) at 66.7%, Columnar Cell Hyperplasia at 16.7%, CCC and CCH occurring together at 16.7%. No case of flat epithelial atypia (FEA) variant was found.

This study shows that CCLs occur among Nigerian women and by extension the Black women population. The FEA variant, the putative DCIS precursor, was not found among our cohort, probably because of the restriction of study material to fibrocystic changes. The age distribution of our patients was similar to those reported among Caucasian women. We recommend that larger studies be carried out when mammography becomes more widely available.

P55

Molecular Subtypes and Prognosis in Two Cohorts of Norwegian Breast Cancer Patients

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Purpose: Our group previously subtyped tumours from a historic cohort of Norwegian breast cancer patients born 1886-1928, with limited access to adjuvant treatment and mammography screening. The purpose of this study was to compare the distribution and prognosis of these subtypes with a second cohort of breast cancer patients born 1917-1972 from the same geographical area.

Methods: Tissue microarrays were constructed from archival formalin-fixed, paraffin-embedded tissue from 909 (Cohort 1) and 514 (Cohort 2) cases. Using IHC and ISH as surrogates for gene expression analysis, tumours were classified into six molecular subtypes: Luminal A, Luminal B (Her2 negative), Luminal B (Her2 positive), Her2 type, 5 Negative Phenotype (5NP) and Basal-like phenotype (BP). Proportional hazards models were used to assess prognosis.

Results: Mean age of diagnosis was higher in cohort 1 (72.5 vs 58.3 years). 47.6% (Cohort 1) and 52.9% (Cohort 2) of tumours were Luminal A. Survival increased for all subtypes in cohort 2. For the first five years after diagnosis, risk of death from breast cancer was highest for Her2 type and 5NP in cohort 1 (age-adjusted hazard ratio 4.4 and 3.2 when compared with Luminal A), and for BP and Her2 type in cohort 2 (age-adjusted hazard ratio 10.5 and 7.5). Mean age for BP cases was lower in cohort 2 (53.2 vs 71.7 years). After the first five years no significant differences in risk of death were found between subtypes.

Conclusion: Cohort 2 comprised younger women with a higher proportion of Luminal A tumours, and with improved survival for all subtypes. BP appears to imply a relatively poorer prognosis among younger women compared to older women. Molecular subtyping gives prognostic information the first five years after diagnosis.

P56

A Pathological Review of Parathyroid Carcinoma

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Background: Parathyroid carcinomas are rare tumours with incidence of 0.5-4% of all the cases of primary hyperthyroidism. The Royal College of Pathologists (RCPath) minimum dataset (2010) has identified a number of features which help in establishing a diagnosis of malignancy. The current study aims to review all parathyroid carcinomas diagnosed at Nottingham University Hospital and Sherwood Forest Hospital, Sutton-in-Ashfiled, between 2004 and 2014.

Material and Methods: Pathology computer databases were searched at both hospitals to identify all parathyroid carcinomas diagnosed during study period. Reports were assessed against RCPath minimum dataset items suggestive of malignancy.

Results: Overall 9 patients were diagnosed as parathyroid carcinoma (7 females, 2 males). The age ranged from 29 to 70 years (median 56years). Macroscopically, specimen weights ranged from 0.7g to 27.5g. The tumour size varied between 15mm and 46mm (median35mm). Documentation of various features of malignancy was as follows; thick fibrous capsule 3/3, dense fibrous septae 7/7, diffuse sheets 3/3, nuclear monotony 3/4, frequent mitoses (>1 per 10hpf) 2/2, vascular invasion 7/9, peri-neural invasion 0/9, direct extension into adjacent soft tissues 7/8, capsular invasion 7/7. Predominant growth patterns were; diffuse sheets (4), irregular islands (2), trabeculae (1), lobules (1) and solid (1). Ki-67 labelling index (reported in 4 cases) ranged from 1% to 3.9%. None of the cases showed abnormal mitoses.

Conclusion: This review shows that there is room for improvement in documentation of features of malignancy in parathyroid carcinoma. We are reviewing the original slides to correlate histological features with current RCPath dataset items.

P57

Snomed Code M09350; 'Morphological Description Only' – A Six Month Review

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Purpose of the study: The SNOMED coding system is used to designate T (Topography), M (Morphology) and P (Procedure) codes. This system is invaluable in the audit of workload and as such accurate coding is imperative. The aim of this review was to examine the usage of the morphological code M09350, designated as a "morphological description only", over a six-month period within a single histopathology department. The objective was to assess the appropriateness of cases for which this M Code was designated.
Methods: A computerized search was performed and the authorized cases coded with the M code 09350 were reviewed. Descriptive reports, without definitive diagnosis were deemed appropriately coded. Cases were deemed inappropriate if an alternative specific M code was available.
Summary of results: In total 160 cases were coded with M09350 in the six month period. 53% of cases were deemed to be inappropriate. 10% of inappropriately coded reports described reactive or regenerative changes. 11% of the coded specimens were limbs referred to the department for safe disposal. 7% of cases described squamoproliferative lesions. With 1% comprising thyroid cytology specimens and 2% bone marrows showing a myeloproliferative disorder. The more commonly coded specimens included skin (28%) and bone marrow (13%), reflecting the fact that reports from these sites commonly require descriptive commentaries with a definitive diagnosis depending on clinicopathological correlation.
Conclusions: The morphological code m09350 should be used for specimens where a morphologically descriptive report is issued and no specific diagnosis is made. Coding can be improved by education of pathologists and introduction of more specific codes in problem areas such as thyroid cytology, disposal cases, myelodysplasia, squamoproliferative lesions and regenerative changes.

P58

When Are We Picking up the Phone? An Audit of Critical Value Reporting

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Purpose of the Study: In 2013 The Royal College of Pathologists issued guidelines on the communication of urgent reports or unexpected findings. Numerous studies have looked at the concept of critical value reporting in pathology however it has proven to be a difficult model to apply to the myriad of pathological diagnoses. This audit aimed to assess the cases which were communicated directly to the clinical teams.
Methods: Computerised laboratory database was retrospectively reviewed for reports coded as having been phoned directly to clinicians. All such reports were examined to identify clinical features, diagnosis and comments which may indicate a critical or unexpected diagnosis.
Summary of Results: 607 telephoned reports were identified in the interval between January 2011 and August 2014, out of a total of 114320 cases. Malignant cases accounted for 57.3%, frozen sections for 9.7%, and cytology for 7.7%. While the majority of calls related to the routine communication of results and requests for clinical information, 25 unexpected or critical events were identified. These included 5 cases of delayed malignant diagnosis, 5 cases of frozen/permanent disagreement, 4 products of conception without villi, 3 cases of vasculitis, 2 cases of suspected perforation with fat in a biopsy sample, and 2 cancers upstaged following additional levels.
Conclusions: This audit showed that 0.5% of cases were telephoned directly to clinicians, this may be an under representation as the coding system is user dependent. Establishing local guidelines on critical cases may facilitate more efficient communication of urgent and unexpected results to our clinical colleagues.

P59

Mediastinal Biopsies: Small Biopsies for Challenging Cases – A Single Centre Review

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Mediastinal masses comprises a varied and mainly neoplastic lesions arising from intra-thoracic organs. In 2011 guidelines for the interpretation of mediastinal biopsies have been published but it still remains challenging. We present data from a review of the mediastinal biopsies reported in the last two years within our department.
We have reviewed 95 consecutive mediastinal biopsies from January 2012 to December 2014. We compared each case preliminary and final diagnosis after performing special stains, molecular analysis or referring the case. Also, when additional material was available, we compared the diagnosis of both samples to find any discrepancy, especially when the first specimen was sent for frozen section examination. For those cases with no available follow up, we reviewed the slides to assess the accuracy of the diagnosis.
46 out of 95 cases had further material for comparison and there was no discrepancy between the two diagnoses. 34 frozen section reports were all confirmed on paraffin sections. 49 cases had no further material available for diagnosis, however 17 were sent for expert opinion and our diagnosis was confirmed. The remaining 32 cases were reviewed by two pathologists and there was independent agreement in all cases. 12 cases out of 95 showed no discrepancy in the diagnosis after review, however they were considered overall not diagnostic after comparison with further material: 2 cases were frozen sections with resection specimen immediately available, 10 cases were small biopsies and additional tissue was sent within few weeks. 6 cases were not diagnostic because insufficient amount of tissue (3 to 15mm) resulting in difficult processing whilst sampling error occurred in the other 6 cases.
Mediastinal masses often pose a diagnostic challenge for pathologists. Our data show that a correct diagnosis is often reached on biopsy (87.4% viewed as adequate). However good sampling is essential as non-diagnostic/inadequate levels were 12.6%.

P60

Investigation of a Rapid Reliable Method for Tumour Percentage Estimation for Diagnostic Molecular Analyses

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Background: Tumour percentage estimate (TPE) is important index for deciding adequate tumour presence in tissue sections submitted for diagnostic molecular analysis. The commonly employed crude visual (microscopic) estimate method is rapid but subject to inter-observer variation and may be grossly inaccurate for tissues with sparse tumour population. Stereological counting methods are reliable but can be labour intensive and time consuming.
Aim: Investigation of reliability and time efficiency of stereological grid point counting method for routine tumour percentage estimation.
Methods: A point lattice (25 dots x 52 dots 0.8cm apart) outlined on a transparent sheet was superimposed on a desktop screen projecting the respective Aperio digital images (x20 mag) of H & E stained sections of 5 (A-E) different colorectal cancers. TPEs based on counts of tumour and stromal nuclei touching the point lattice at every 5th (5TPE) or 10th (10TPE) line were derived for a fixed area (41x19.8 cm) and compared with absolute TPE (ATPE) and crude TPE (CTPE).
Results: The CTPE and ATPE, 5TPE5 and 10TPE for the 5 cases (A-E) were calculated to be as follows, respectively: A — 40, 49.3 53.3, 54.1; B — 65, 62.5, 57.3, 57.1;; C — 60, 71.7, 73.7, 73.8; D — 70, 72.6,74.8, 73; E — 90, 89.9, 91.2, 90.6. The average time taken to obtain these 2, 30, 7.5, 15 min, respectively.
Conclusion: The lattice grid point counting is potentially more reliable the crude visual for tumour percentage estimation, meriting investigation of a grid point lattice directly superimposed on the microscopic image as a more direct alternative stereologically sound cell counting method.

P61

Ensuring Quality in Histopathological Reporting of Laparoscopic and Open Colorectal Cancer Resections

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Purpose: With increased use of laparoscopic procedures over open surgery in colorectal cancer resection we need to ensure that pathological reporting standards can still be met with specimens from laparoscopic surgery, even with smaller mesenteric samples. We audited the histopathology reporting of laparoscopic and open colorectal cancer resections against the minimum standards in The Royal College of Pathologists (RCPath) published dataset guidelines.
Methods: Patients who underwent colorectal resection for malignancy over a twelve month period were identified through clinical coding. The list was verified using the pathology report and incorrectly coded patients excluded. Laparoscopic procedures were identified and an equal number of open resections were randomly selected. Pathology reports were analysed as to; type of resection, number of lymph nodes examined, serosal involvement, tumour stage, extramural venous involvement. Differences between the two groups were analysed using a Student's t-test. A significance level of p<0.05 was used throughout.
Results: Thirty seven lapraroscopic resections and 35 open resections were analysed. A greater proportion of right hemicolectomies were laparascopic. Mean number of lymph nodes sampled for laparoscopic and open procedures were 16.3 and 17.1 respectively. Serosal involvement was 17.6% and 16.1%. Incidence of extramural venous invasion was 40% for both. No significant difference was noted between the groups.
Conclusions: Although the sample sizes are small, there was no difference between laparoscopic and open surgery in meeting the RCPath standards. This supports the increased use of laparoscopic surgery in colorectal cancer. There is a lower incidence of serosal involvement in these resections than that recommended in the dataset. This may be due to earlier detection of tumours following the introduction of the Bowel Cancer Screening Programme.

P62

An Audit of Malignant Melanoma Histopathology Reporting After Proforma Introduction

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Purpose of the study: This audit's objective was to determine whether malignant melanoma reporting was including the core criteria as expressed in the minimum dataset requirements of the *Royal College of Pathology Dataset for the Histological Reporting of primary cutaneous malignant melanoma (3rd Edition)* and if this had improved since the introduction of a compulsory proforma.
Methods: All histological diagnoses of malignant melanoma at the hospital over two 12 month periods were identified using SNOMED codes on the pathology database pre and post the compulsory proforma.
Summary of results: Within the hospital a reporting proforma is available with the microscopic core-criteria. During the year a national electronic proforma has been introduced including both macroscopic and microscopic core criteria. 93% of reports used a proforma compared with 66% in the previous study with an overall improvement of core-criteria inclusion from 69% to 93% as well as 70% of reports now including TNM staging compared with 0% previously. Overall there was a discrepancy between the inclusion of macroscopic (87%) and microscopic (97%) core criteria.
Conclusions: There was a significant improvement in the overall core-criteria inclusion percentage since making proforma use compulsory. There is still room for improvement when reporting macroscopic criteria, the use of the national proforma, (which includes this and had started to be used towards the end of this study) aims to improve this.

P63

Educating in Healthcare – How we can do it Better

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There is evidence that the quality of education and the environment in which it occurs in the NHS are in need of improvement (Francis, Berwick Reports). The UK General Medical Council reports tensions between "service" and "education/teaching/training". An educational environment is the relationship of learning with the people who are learners in their physical temporal and social surroundings. Do we attend to the educational environment to provide learners and teachers with the best outcomes for sustainable laboratory services? We suggest not.
This paper is a synthesis of data from multi-disciplinary workshops in conferences (London, NACT; Milan, AMEE) in 2014, involving 59 and 34 participants respectively. These were doctor-educators and academics with pedagogical interest. It also draws on a discrete choice experiment (DCE) with 1323 trainee participants surveyed across specialties in Scotland and the north of England in autumn 2013.
Workshop data demonstrate several themes. Problems include a dichotomy between the accepted "continuum of education" and processes involved in its parts. Employers' expectations do not match those of trainees. It is hard to translate educational ideas into practice, relying on individuals who succeed despite rather than because of institutional support. Medicine relies on communication and human interaction: educational relationships are challenged and role models fail to invigorate and enthuse. Solutions encompass the need to generate a culture in healthcare that values, normalises and prioritises education. Fostering educational relationships and conscious socialisation may facilitate this as may transparency in funding. The DCE highlights the importance trainees place on good working conditions while making career choices, above good programmes and nice places.
In considering future diagnostic services, it may be worthwhile to note the evidence and have the people placed in an educational culture at the heart of plans.

P64

Investigation of the Role of Alcohol in Intestinal Cancer

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Background: Alcoholic beverages have been classified as carcinogenic, with tumour sites related to alcohol consumption including the colorectum and upper aerodigestive tract (IARC 2007). A variety of mechanisms may be causal for the enhanced cancer risk linked to alcohol consumption, including direct genotoxic effects of ethanol and its metabolite acetaldehyde (AA). The relevance of these mechanisms in the colorectum has not been fully elucidated yet, neither has the role of different isoforms of alcohol metabolising enzymes. While Aldh2, the main enzyme for AA detoxification in the liver, has been investigated widely, little is known about Aldh1b1 which also has a high affinity for AA and is highly expressed in the GIT.
Aims: We aim to further elucidate the mechanisms of alcohol as a risk factor for cancer in the intestines, focussing on alcohol metabolising enzymes and DNA repair, and on genotoxic effects.
Methods: 20% (v/v) ethanol was administered to mice via drinking water for 3 weeks, while control groups received normal drinking water. Swiss rolls of the intestine were prepared for immunohistochemical analysis and intestinal epithelium was isolated.
Results: While Aldh2 was uniformly expressed in the intestinal epithelium, Aldh1b1 expression was preferentially located in the crypt bases. Cyp2e1 was up-regulated by alcohol in the liver, but not expressed in the intestines. Alcohol increased the number of Ki67-positive cells in the liver and small intestine. Enhanced phospho-γH2AX and p53 were observed in the colon, indicating DNA damage.
Conclusions: We have established a protocol for short term treatment of mice with ethanol and we demonstrated DNA damage and enhanced proliferation after 3 weeks of ethanol treatment. This provides a good basis for further elucidation of the genotoxic mechanisms of alcohol and its metabolites, as well as the protective mechanisms involved.

P65

This abstract has been withdrawn

P67

Clinicopathological Correlations of ETS1 Protein Expression in Urothelial Carcinoma of the Urinary Bladder

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Background: ETS-1 proto-oncogene is an important transcription factor that plays a role in the regulation of physiological processes, such as cell proliferation and differentiation. ETS-1 was previously shown to play a substantial role in the proliferation and invasion of tumour cells in many types of solid cancers by enhancing the transcription of matrix metalloproteinases and urokinase-type plasminogen activator.
Aim: To investigate the expression pattern of ETS-1 protein in urothelial carcinoma of the urinary bladder and determine its potential association with the well-established prognostic histopatholoical parameters, including tumour grade, stage and lymphovascular invasion.
Materials: Sixty five specimens of urothelial carcinoma and a total of six normal urothelial tissues were stained immunohistochemically with anti-ETS-1 monoclonal antibody and scored by two hisopathologists.
Results: The normal urothelium showed weak scattered ETS-1 expression, with few weakly stained stromal cells. All tumours showed a degree of positivity of ETS-1 cytoplasmic expression, however; with variable degrees. Interestingly, high grade tumours and invasive tumours to the lamina propria and muscle had significantly higher ETS-1 expression than low grade tumours and the non-invasive ones. ETS-1 expression showed a strong positive correlation with the tumour grade (P<0.001), however, showed no correlation with neither with tumour stage nor lymphovascular invasion.
Conclusion: ETS-1 protein expression is significantly associated with high grade tumours in urothelial carcinoma of the urinary bladder; therefore, the up-regulation of ETS-1 expression could have a potential role in carcinogenesis and differentiation in such malignancies.

P66

Cytokine Networks in Endometrial Carcinogenesis

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Endometrial cancer (EC) is the commonest gynaecological malignancy in the developed world and falls into two categories. Type I (~75% cases) is generally oestrogen-sensitive, develops from premalignant hyperplasia and is low-medium grade. By contrast, the rarer high grade Type II is frequently oestrogen receptor negative, have no premalignant lesion and have poorer outcomes. This study characterised cytokine-based microenvironmental features associated with endometrial carcinogenesis given their role in immunoregulation and tumour behaviour.
Endometrial lysates (38 normal, 25 hyperplastic and 97 cancerous; 46 Type I, 51 Type II) were profiled for 49 cytokines by multiplex immunoassay which were then standardised against total protein. Data were analysed by Kruskall-Wallis tests with Mann-Whitney-*U* tests *post hoc*, applying False Discovery Rate correction for multiple comparisons. The *R* package *Catnet* was used for learning categorical Bayesian networks, where data were fitted according to Maximum Likelihood Estimation-based network search by Simulated Annealing without a prior seed network. Final networks were selected based on maximal Akaike information criterion values and visualised in Gephi.
Significant differences in the concentration of 28 cytokines were noted between the groups. Cytokine profiles matched the robust histology-based discrimination between normal, hyperplastic and cancerous endometrial tissues, including differentiating between Type I/II cancers. These differences were also reflected in cytokine interrelationships; distinct subnetworks with different nodal foci were seen across all groups. In particular, GM-CSF appeared to play a major regulatory role uniquely in normal endometrium. However, the hub node function of other mediators was more conserved: IL-17 was consistently found in this capacity in all categories except Type II ECs, which instead favoured IL-4 and IFN-γ. Moreover, a central role for agents such as TNF-β was only seen in cancers.

P68

Histopathologic Assessment of Testicular Specimen in a Nigeria Tertiary Hospital

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Introduction: Removal of testicular tissue is often performed for the evaluation or treatment of testicular lesions. Histopathologic assessment of such specimen is therefore useful in the review of current urological practice. Testicular biopsies are also of vital importance in the management of infertility.
Aim and objectives: This study aims to characterize the histologic spectrum of testicular lesions as well as relating them to the various surgical procedures by which they were taken.
Methodology: Records of all testicular specimen received in the department from 2005 to 2014 were retrieved. Data such as age, indication for biopsy, nature of surgical specimen and histologic diagnoses were extracted. They were classified, analyzed and represented in tables and charts.
Results: A total of 167 testicular and paratesticular specimens were submitted during the study period constituting 0.7% of surgical specimen received during the study period. The most common indication for the submission of testicular specimen was for the treatment of prostate cancer (43.7%) followed by pain (20.8%) and the presence of a mass (19.6%). Orchiectomy specimens were the commonest samples received (76.8%). A significant proportion of cases 56.25% had orchiectomy for benign lesions. Hypospermatogenesis with maturation arrest (57.8%), hypospermatogenesis (15.8%) and tubular hyalinization (15.8%) were the common histologic diagnosis of male infertility. Germ cell tumours were the commonest testicular neoplasms (62.5%). Embryonal rhabdomyosarcoma was the only paratesticular malignancy seen.
Conclusion: Treatment of prostate of cancer was the commonest indication for testicular biopsies in our environment. Testicular tumours are not common. Fifty six percent of the cases had orchiectomy for benign lesions which call for a review of practice. With core needle biopsies and frozen section analysis, unnecessary orchiectomies can be avoided.

P69

An Interesting Case of Persistent Mullerian Duct Syndrome-Rare Female Variant

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Persistent Mullerian duct syndrome is a rare form of internal male pseudohermophroditism characterised by the presence of Mullerian duct derivatives such as cervix, uterus and tubes in a phenotypically and karyotypically male patient (46,XY). Only 150 cases have been reported in the literature so far with two types of variants described. The more common male form accounts for 80-90% of the cases and is characterized by unilateral cryptorchidism and contralateral inguinal hernia containing uterus and fallopian tubes. The female form accounts for only 10-20% the cases where in there is bilateral cryptorchidism with the testis fixed within the round ligaments in an "ovarian" position inside the pelvis.
We report a 49-year-old gentleman diagnosed with the rare female variant of persistent Mullerian duct syndrome. He presented with bilateral cryptorchidism in childhood and was further lost to follow up. He re-presented with symptoms of left ureteric calculus and underwent a computed tomography. This demonstrated a saccular structure anterior to the bladder with bilateral tubes leading onto a pair of intraabdominal gonads. Further investigations revealed a 46,XY karyotype in the gentleman. A surgical excision of the remnant structures was then performed with histopathology confirming the presence of primitive cervix and uterus along with a pair of fallopian tubes representing Mullerian duct remnants. The gonads present in the "ovarian" position were confirmed to be testes exhibiting features of testicular dysgenesis and changes secondary to cryptorchidism. A pair of spermatic cord ran alongside the fallopian tubes in the same sheath and a pair of seminal vesicles on either side of the primitive cervix was also found. To have such well-formed male and female genital organs running alongside each other interconnected as in this case is believed to be rare.

P70

Re-audit of Prostate Needle Core Biopsy (NCB) Reports with Adenocarcinoma

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Purpose of study: Re-audit of an original project performed in 2011 which assessed reporting of prostate NCB with adenocarcinoma. The results from 2011 showed that although there was good compliance for reporting important prognostic criteria (dictated by the RCPath dataset), there was variability between different pathologists within the institution. The main recommendation was to use an agreed standard template for all NCB specimens with cancer to ensure consistency in reporting so that high standards were maintained. This re-audit will evaluate whether the recommendations have been met and whether this has improved reporting standards.
Methods: A retrospective computer search identified all prostate NCB containing adenocarcinoma from October 2011 to October 2013. Fifteen reports by each consultant were then randomly chosen from the list for assessment (105 total). Each report was examined against the following criteria:
• Specimens submitted according to local protocol
• Use of standard template
• Grouping of cores according to side of prostate
• Localisation of cores involved with cancer: apex, mid or base
• Gleason Score given
• Correct terminology used
• Reporting tumour extent: Number of cores involved by adenocarcinoma as a fraction of total number submitted from that side; total percentage cancer involving each side
Results: 100 % compliance was achieved in 6/8 variables. Improvement in compliance with all criteria was also seen.
The two variables where the results were short of 100% were:
• Use of standard template; (99% achieved): An individualised report was required in one case where material did not survive processing.
• Specimen submitted according to local protocol; (96% achieved - 4 cases)
Conclusion: The original audit recommendation of using a standard template for prostate NCB has been implemented well and and has provided significant improvement in reporting standards. Important prognostic criteria are now included with less variability and greater consistency.

P71

Audit of the Reporting of Testicular Neoplasms within a University Teaching Hospital

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An updated dataset guiding the reporting of testicular neoplasms was issued by the Royal College of Pathologists in May 2014. This defined core items that need to be represented in all histological reports of testicular neoplasms. Several of these core dataset items were adjusted from previous dataset published in October 2007. Examples of these adjusted items included rete stroma and hilar soft tissue invasion and documenting which tumour type showed lymphovascular invasion in mixed germ cell tumours.
All testicular neoplasm reports from a tertiary centre within an 18 month period from July 2013 to December 2014 were analysed. A total of 67 cases were identified of which were 61 germ cell tumours and 6 were non germ cell tumours. Compliance of the core items were analysed by comparing report contents with the Royal College of Pathologists guidelines operational at the time of reporting.
There was good compliance with items such as type of tumour 61 (100%), lymphovascular invasion 61(100%), intratubular germ cell neoplasia 61(100%). rete stromal invasion was mentioned in 29/31(94%) of cases reported after introduction of the new dataset. There were areas for improvement, including the reporting of core data items that have remained unchanged from previous guidance, such as maximum tumour diameter 57/61(93%) and spermatic cord invasion 48/61(78%).
Identification of such areas for improvement allows action to be taken to ensure that pathology reports meet the minimum standards recommended by the Royal College of Pathologists.

P72

Audit of Seminal Vesicle Invasion in Radical Prostatectomy Specimens, Do We Really Need to Process the Lot?

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Purpose of the Study: Seminal vesicle invasion (SVI) in radical prostatectomy specimens has a poor prognosis and is staged pT3b in the UICC 7th edition. No universally accepted guidelines currently exist on the embedding of seminal vesicle (SV) tissue. We aimed to audit our incidence of pT3b reporting and in these cases to look at SVI-type (Type 1-3) and SV sampling method, with a view to moving from complete to partial embedding.
Methods: 258 radical prostatectomy cases over a four year period were identified. These specimens were entirely embedded. Cases reported as pT3b were re-reviewed to confirm SVI and the SVI-type documented.
Results: 16 of 258 cases were reported as pT3b (6.2%). Of the 14 cases where it was possible to determine SVI-type, 13 cases (92.8%) showed type 2 invasion and in all of these cases invasion was confirmed in either the slice of the junction of the base of prostate and SV (junctional slice) or the most proximal SV section. In the one case (7.2%) of type 3 invasion the proximal and mid SV and the junctional slice were negative for tumour invasion. However, lymphovascular invasion (LVI) was identified in the prostate in this case and confirmed with immunohistochemistry. Block numbers for SV tissue averaged 5.6 regular blocks or 1.5 megablocks per case.
Conclusion: Our incidence of stage pT3b disease of 6.2% is within the expected published range (6% - 19.3%). Our results support a partial SV embedding technique sampling only the junctional slice or the most proximal slice of the SV, rather than mandatory blocking of all the SV tissue routinely, as tumour infiltration of the mid and distal SV is rare in the absence of involvement of the proximal SV. However, the entire SV should be retrospectively blocked if LVI is identified in the prostate. Partial embedding should reduce costs and could also improve turnaround times.

P73

Case review: Birt-Hogg-Dubé Syndrome

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Birt-Hogg-Dubé (BHD) syndrome is a rare autosomal dominant genetic disorder arising from germline mutations in the folliculin gene, mapped to the short arm of chromosome 17. It is associated with cutaneous lesions including fibrofolliculomas, spontaneous pneumothorax and renal tumours. We present the case of newly diagnosed BHD syndrome in a 45 year old Irish female. Histological examination confirmed multiple chromophobe renal cell carcinomas (RCC) and a hybrid oncocytoma/chromophobe RCC. There was also background oncocytosis, comprising multiple, tiny parenchymal micronodules of clear cells with chromophobe RCC-like morphology and immunophenotype. Mutation of the folliculin gene was confirmed by direct sequencing.

Pathologists should be aware of the possibility of BHD syndrome in patients presenting at a young age, with multiple renal tumours with either chromophobe RCC, oncocytoma or hybrid oncocytoma/chromophobe RCC features, particularly if there is also background oncocytosis. This case is illustrative of the fact that chromophobe RCC-like cells with clear rather than oncocytic cytoplasm form part of the oncocytosis spectrum and this finding should always prompt appropriate genetic analysis for BHD syndrome

P74

Defining the Rectum and Sigmoid Colon: Should We Abolish the Term 'Rectosigmoid' to Improve Outcomes in Colorectal Cancer?

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The risk of circumferential resection margin (CRM) involvement is confined to tumours of the rectum with the risk of peritoneal involvement increasing the further a tumour is located above the peritoneal reflection. There is no internationally accepted definition of the upper limit of the rectum, and the term 'rectosigmoid' is frequently applied to tumours in this area leading to confusion around the risks and whether radiotherapy can be given.

The photographs from 331 abdominoperineal excision specimens were available for quantitation using Aperio ImageScope. Both fresh and fixed specimen images were included where available. The position of the anal verge, top of the sphincters, anterior peritoneal reflection, mesorectal apex (defining the limit of the mesorectum) and high vascular tie were identified and the distances between each point measured. The work was supported by a PathSoc bursary.

There was wide variation in the length of the mesorectum in both fresh (median 172 mm, IQR 146 to 199 mm) and fixed (166 mm, 140 to 196 mm) specimens. The length of the anal canal also showed variation (fresh 66 mm, 49 to 78 mm; fixed 66 mm, 53 to 75 mm). The height of the anterior peritoneal reflection was lower in females compared to males (fresh 125 vs. 132 mm, p=0.288; fixed 111 vs. 126 mm, p=0.034).

There is marked variability in the anatomy of the rectum between individuals and genders. This potentially affects the risk of either CRM or peritoneal involvement and whether radiotherapy could be offered. A fixed definition of the upper limit of the rectum for all patients is not helpful. This should be determined for individual patients on the basis of the MRI findings. The term 'rectosigmoid' should be abolished and more accurate definitions based on the position of the mesorectal apex and commencement of the sigmoid mesentery should be used to define the boundaries of the rectum and sigmoid colon and determine subsequent risks to the patient.

P75

Pre-Treatment and Post-Treatment Epidermal Growth Factor Receptor Pathway Mutations in a Prospective Phase II Trial (NWCOG EXCITE) of Cetuximab-Containing Chemoradiation in Locally Advanced Rectal Cancer

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Pre-operative chemoradiotherapy (CRT) with anti-EGFR antibodies may change the status of EGFR pathway mutations. We assessed the mutational status of a number of EGFR pathway genes before and after CRT in the NWCOG EXCITE trial.

Patients with MRI-threatened surgical margins were given pelvic radiotherapy (45Gy) with capecitabine, irinotecan and cetuximab followed by surgery after 8 weeks. DNA was retrospectively extracted from the pre-treatment biopsy and resection specimen by macrodissecting areas of greatest residual tumour. The mutational status of KRAS (codons 12/13/61/146), NRAS (12/13/61), PIK3CA (542/545/546/1047) and BRAF (V600E hotspot) were determined by pyrosequencing. The work is presented on behalf of the NWCOG EXCITE trial investigators and was part-funded by a PathSoc fellowship.

80 patients commenced treatment and 76 underwent surgery with pathological complete response in 14 (18%) and near-complete in 6 (8%). Pre-treatment testing (n=78) detected mutations in KRAS (n=34), BRAF (n=3), NRAS (n=3) and PIK3CA (n=10). Any EGFR pathway mutation was detected in 58%. Following CRT, cases with residual tumour able to be tested (n=54) showed mutations in 32 patients (59%). There was a discrepancy compared to pre-treatment biopsy in 18 cases (33%): from wild-type (wt) to mutant (mut) in 9, from mut to different mut in 1 and from mut to wt in 7. One patient changed in 3 codons (mut to wt in KRAS 146/PIK3CA 545 and wt to mut in KRAS 12). In 12 patients (22%) this changed their overall EGFR pathway status (6x wt to mut and 6x mut to wt).

Intratumour heterogeneity may explain some of the differences in EGFR pathway mutations reported between biopsies and resections presenting a challenge to personalised medicine. However, cetuximab may also drive the growth of undetectable mutant clones to detectable levels on pyrosequencing. Further assessment using more sensitive sequencing technologies is currently being employed to investigate these differences.

P76

Investigating the Challenges of Using Historical Formalin-Fixed Paraffin-Embedded (FFPE) Material from the MRC CR07 Rectal Cancer Trial Using the Affymetrix OncoScan® FFPE Assay and Next Generation Sequencing

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There is a vast amount of historical FFPE material held in archives, but due to variations in fixation and processing this presents several challenges when applying newer genomic technologies to it. In this study we compared the genomic information obtained with the OncoScan® FFPE Assay Kit (OncoScan) and next generation sequencing (NGS).

Samples from 378 patients were obtained from 10 centres taking part in the MRC CR07 trial of short course radiotherapy versus selective long course chemoradiotherapy in rectal cancer. DNA was prepared using Agilent SureSelect kits and sequenced using Illumina platforms in parallel to analysis using the OncoScan assay. For both methods, quality control (QC) data was generated and the sample classified as a 'pass' if it fell within the pre-defined QC boundaries. For the OncoScan assay, copy number (CN) and somatic mutation (SM) data was further investigated. This study was part funded by a PathSoc Fellowship.

In total, 272 cases (72%) passed the NGS QC and 232 (61%) passed the OncoScan QC. A total of 186 (49%) passed QC on both platforms with marked variability in sample pass rates between the 10 centres for the NGS (range 0% to 100%) and OncoScan (ranges 33% to 84%). When assessed manually, the OncoScan SM data was considered acceptable for 273 cases (72%), which included 40 initially classified as 'failed' by the QC data. Similarly, the OncoScan CNV data was interpretable for the majority of cases. This study has shown that whilst historical DNA held in the FFPE blocks of archival clinical trials like MRC CR07 can present challenges when using new genomic technologies, a large proportion of samples can still yield valuable genomic data. Marked variation exists in the quality of genomic material between centres confirming that differences in specimen handling affect DNA quality. Prospective trials must address this by standardising fixation and processing protocols.

P77

The Effect of Multidisciplinary Education on the Quality of Colon Cancer Resection

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The plane of colon cancer resection has recently been shown to predict survival. Complete mesocolic excision (CME) with central vascular ligation (CVL) produces an oncologically superior specimen and appears to be related to optimal outcomes. We aimed to assess whether a regional educational programme in CME with CVL led to an improvement in the quality of colon cancer specimens.

Following a regional educational programme in CME with CVL in the Capital and Zealand areas of Denmark, 686 cases of primary colon cancer resected across six hospitals were assessed by grading the plane of surgery and undertaking tissue morphometry. These were compared to 263 specimens resected prior to the educational programme. This work was partly supported by a PathSoc undergraduate bursary.

Across the region, the mesocolic plane resection rate improved from 58% to 77% (p<0.0001). Hillerød hospital had implemented CME with CVL as standard prior to the educational programme and continued to produce optimal specimens. Three of the other hospitals showed a significant improvement in the plane of surgical resection. Hillerød specimens continued to be more radical with a greater distance between the tumour and the high tie, area of mesentery and lymph node yield compared to the other five hospitals.

A multidisciplinary regional educational programme in CME with CVL has improved the oncological quality of colon cancer specimens as assessed by mesocolic planes, however, there has been no significant effect on the amount of tissue resected. Surgeons at Hillerød continue to produce more radical specimens suggesting that such educational programmes are not alone sufficient to increase the amount of tissue resected around the tumour. Hillerød have recently published their long term outcomes with survivals being 10% higher when compared to other hospitals across the region. Further engagement is now necessary to ensure that optimal outcomes are achieved across the region.

P78

Investigating the Faecal Microbiome in Formalin Fixed Paraffin Embedded (FFPE) Material

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Purpose: Research into the faecal microbiome has shown a diverse population with a high level of variability between individuals. Altered faecal microbiomes are present in a range of diseases but work remains to understand their role in gastrointestinal disease. Current research into the microbiome makes use of fresh or frozen faecal samples. This restricts researchers to predominantly prospective study designs. One potential method for rapidly increasing and diversifying research is the retrospective study of FFPE material. We aimed to investigate the feasibility of typing the microbiome in FFPE faecal samples using next generation sequencing (NGS) technology.

Methods: Material from six faecal samples was divided and stored as frozen or fixed and paraffin embedded creating two matched sub-groups. To assess assay sensitivity one sample was diluted to eight different concentrations before fixing and embedding. The V4 and V6 regions of the 16s rRNA gene were amplified. Primer pairs created approximately 240bp and 98bp targets in E.coli respectively. PCR products were multiplexed and sequenced on an Illumina MiSeq. QIIME software was used for analysis.

Results: Analysis of alpha (within sample) diversity showed a significant difference between sub-groups when targeting V4 (p=0.05) but not V6. Analysis of beta (between sample) diversity showed a significant difference between sub-groups when targeting V4 (p=0.01) while the V6 region showed a reduced, but still significant (p=0.02) difference. The sensitivity assay showed comparable results down to 0.5% concentration levels.

Conclusion: To our knowledge this is the first feasibility study generating NGS data on the microbiome from FFPE faecal material. Variation between matched frozen and FFPE faecal material was less when targeting V6 compared to V4. We hypothesise this may be due to the shorter amplicon undergoing less DNA fragmentation in FFPE material.

P79

Comparison of Mutational Calls Obtained with Pyrosequencing and the Affymetrix OncoScan® FFPE Assay in Patients with Colon Cancer Recruited to the NCRI FOxTROT Trial

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There are several platforms available for DNA mutation detection in formalin-fixed paraffin-embedded (FFPE) material, all with their relative strengths and weaknesses. We investigated the OncoScan® FFPE Assay Kit (OncoScan) in comparison to pyrosequencing in patients with operable colon cancer recruited to the phase II component of the NCRI FOxTROT trial of pre-operative vs. post-operative chemotherapy.

FFPE samples of tumour from the resection specimens of 132 cases were tested for KRAS 12/13/61 and BRAF V600E mutations using pyrosequencing. The OncoScan assay allows for the interrogation of 74 mutations across nine genes. Pre-extracted DNA was analysed on the OncoScan assay and quality control (QC) scores generated, indicating confidence in mutation calling results. The mutational status of all samples was automatically assessed in the Affymetrix SM Viewer, and then manually confirmed. This work is presented on behalf of the FOxTROT Collaborative and was part funded by a PathSoc fellowship.

Out of 132 samples, 22 failed OncoScan QC thresholds, however, only 10 of these were deemed inconclusive by manual interrogation. 130 samples were interpretable by pyrosequencing. Of the 120 samples that produced conclusive results on both platforms, the concordance rate was very high at 95.8% when calling a mutated versus non-mutated KRAS/BRAF status. Mutations were 'missed' by pyrosequencing in only 1 case (0.8%) and by OncoScan in 4 cases (3.4%). In addition, the OncoScan assay provides mutational data in additional genes along with copy number (CN) and loss of heterozygosity (LOH) information.

In patients with colon cancer recruited to the NCRI FOxTROT trial, the OncoScan FFPE assay shows good correlation with pyrosequencing when determining the mutational status of KRAS/BRAF. Although pyrosequencing has a slightly lower failure rate, the OncoScan has the added advantage of targeting more mutations, producing genome wide CN, and LOH information in one assay

P80

Whole-Mount Microscopic Sections Reveal that Denonvilliers' Fascia is One Entity and Adherent to the Mesorectal Fascia: Should we still Question the Anterior Plane in TME?

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Excellent anatomical knowledge of the rectum and surrounding structures is essential for total mesorectal excision (TME). Denonvilliers' fascia (DVF) has been frequently studied, though the optimal anterior plane in TME is still disputed. The relationship of the lateral edge of DVF to the autonomic nerves is also unclear. We studied whole-mount microscopic sections of en-bloc cadaveric pelvic exenteration specimens and describe implications for TME.

Four human adult cadaveric specimens (two males, two females) were obtained from the Leeds GIFT Research Tissue Programme. Paraffin-embedded mega-blocks were produced and serially sectioned at 50 and 250 μm intervals. Sections were stained with haematoxylin & eosin, Masson's trichrome and Millers' elastin. Additionally, a developmental series of eleven human fetal pelvic specimens (embryonic age of 9-20 weeks) were studied.

DVF consisted of multiple fascial condensations of collagen and smooth muscle fibres and was indistinguishable from the anterior mesorectal fascia and the capsule of the prostate or posterior vaginal wall. The lateral edges of DVF appeared fan-shaped, and the most posterior part was continuous with the mesorectal fascia. Peri-rectal fasciae were not identified in fetal specimens.

DVF is adherent to and continuous with the mesorectal fascia. Optimal surgical dissection during TME should be carried out anterior to DVF to ensure radical removal, particularly for anterior tumours. Autonomic nerves are at risk, but can be preserved by following the mesorectal fascia along the anterolateral mesorectum. The lack of evident fasciae in fetal specimens suggests that these might be formed in later developmental stages.

P81

The Anatomy of the Perineal Body in Relation to Abdominoperineal Excision for Low Rectal Cancer

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The perineal body (PB) is poorly understood. In abdominoperineal excision (APE), there is no natural dissection plane through the PB. Knowledge of the PB is essential to avoid straying in to incorrect planes leading to tumour perforation and unnecessary urogenital and anorectal injuries. This study describes the anatomy of the PB and the implications for APE.

Six human adult cadaveric specimens (three males, three females) were obtained from the Leeds GIFT Research Tissue Programme. Paraffin-embedded mega-blocks containing the PB were produced and serially sectioned at 50 and 250 μm intervals. Sections were stained to reveal collagen and elastin, and with an antibody against α-smooth muscle actin.

The PB is formed of a fibromuscular mass, which was thicker and wider in female specimens compared to males, extending from the external anal sphincter to the rectogenital septum. Muscles from the urogenital diaphragm and anterior rectal wall anchored into the PB. The longitudinal muscle (LM) of the rectal muscularis propria extended in anterolateral directions and intertwined with the somatic pelvic floor muscles to create strong fixation of the anorectum.

The LM plays a dominant role in the formation of the PB. Surgeons should be aware of the complex course of the LM through the PB to prevent injuries to the urogenital organs and perforation of the anterior rectal wall. The perineal phase of an APE starts with excellent exposure followed by proper tension on the PB to allow safe dissection through the densely-packed fibromuscular mass.

P82

Impact of Tumour Budding in Oesophageal Adenocarcinomas

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Purpose of the study: Tumour budding has prognostic significance in many carcinomas and is defined as the presence of detached isolated single cells or small cell clusters (up to 5 isolated cells) scattered in the stroma. Tumour budding can be observed at the peripheral invasion front (peritumoural budding=PTB) and/or within the tumour (intratumoural budding=ITB). For oesophageal adenocarcinomas there are currently only few data about the impact of this morphological feature. In the present study we investigated PTB and ITB in a well characterized collective of primary resected oesophageal adenocarcinomas of two centers.

Methods: Whole tissue sections of 201 resection specimens were analyzed. Tumour buds were highlighted by pancytokeratin staining. PTB and ITB were scored across 10-high-power-fields (HPF). Results were correlated with clinico-pathological and follow-up data.

Summary of Results: Interobserver agreement between two independent investigators was substantial to excellent (p < 0.001, intraclass correlation coefficient = 0.77 for ITB and 0.92 for PTB). The median count of tumour buds was 130/10 HPF for PTB (range 2-593) and 80/10 HPF for ITB (range 1-656). PTB and ITB correlated significantly with each other (r=0.9; p<0.001). High PTB/ITB rates were associated with advanced tumour stages (p<.001 each), presence of lymph node metastases (p<0.001/p=0.002), worse tumour differentiation (grading; p<0.001 each) and higher rate of incomplete tumour resection (p=0.003/p<0.001). In addition, PTB was a significant predictor for the presence of lymph node metastases as well as tumour grading (p>0.02 each). Survival analysis showed a trend for an association with worse survival for both high grade PTB (p=0.085) and ITB (p=0.082), but pT and pN category were better predictors for survival (p<0.001 each).

Conclusions: Peripheral and intratumoural budding can be observed in oesophageal adenocarcinomas in various degrees. High grade budding is associated with aggressive tumour phenotype. Assessment of tumour budding may provide additional prognostic information about tumour behavior and may be useful in specific cases for better risk stratification of oesophageal adenocarcinoma patients.

P83

This abstract is not available before the meeting

P84

EBV Positive Mucocutaneous Ulcer with Lymph Node Involvement Causing Sigmoid Stricture – A Case Report

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EBV Positive Mucocutaneous Ulcer (EBVMCU) is a relatively recently described EBV related disease with specific microscopic appearances, immunophenotype and usually good prognosis. Only a small number of cases presenting in the gastrointestinal tract have been described, most associated with iatrogenic immunosuppression.

We present a case of a 74 year old woman with past history of breast cancer 6 years ago treated with wide local excision and radiotherapy, who presented with sigmoid stricture that was clinically considered to be due to diverticular disease requiring sigmoid colectomy.

A deep ulcerating lesion was found at cut-up that was composed of granulation tissue and a dense infiltrate of lymphoid cells, plasma cells and macrophages with intermixed large atypical, Hodgkin/Reed-Sternberg (HRS)-like cells with the following immunophenotype: CD30+, LMP1+, EBER ISH+ and CD15-, CD20-. Some of the lymph nodes harvested from the specimen also showed a para-cortical infiltrate of small lymphocytes with eosinophils and occasional HRS-like cells.

These morphologic and immunophenotypic features are considered to be compatible with EBVMCU with HRS-like cells most likely, in the absence of iatrogenic immunosuppression, secondary to age related immunosenescence. Conservative management was advised.

Full staging investigations were performed which confirmed that the disease was confined to the gastrointestinal tract. She remained asymptomatic seven months after the initial diagnosis.

P85

Genetic Mechanisms In Colorectal Polyposis

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Familial Adenomatous Polyposis (FAP), MUTYH-Associated Polyposis (MAP) and Polymerase Proofreading-Associated Polyposis (PPAP) are syndromes of adenomatous polyposis, and are due to mutations in APC, MUTYH, POLE and POLD1. Patients with multiple colorectal polyps undergo diagnostic analysis of APC/MUTYH and in 50-80% of cases, a mutation is identified. The aim of this study is to identify novel genetic mutations in patients with multiple polyps who have no mutation identified in the known disease-associated genes.

80 patients with ≥ 10 colorectal polyps have been recruited. All are negative for APC/MUTYH mutations following sequence/dosage analysis of coding exons, and patients have been screened for Pol mutations.

Haloplex (Agilent) will be employed for sequence capture of the entire APC/MUTYH genes, followed by ultra-deep sequencing (UDS) on a HiSeq (Illumina). Samples will also undergo cDNA sequencing to screen for allelic imbalance and splicing abnormalities, and qPCR will be employed to assess gene expression.

The characterisation of novel genetic variants associated with polyposis will allow more appropriate clinical management of patients. If the results of this study support the efficacy of this technique over current diagnostic protocols, improving the rate of mutation detection, then it may be readily translatable to clinical practice.

This project has been partly funded by a grant from the Pathological Society.

P86

A 6-Years Review of Colorectal Cancer Resections Reporting as per Royal College Guidelines

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OBJECTIVES: (1) Are we following Royal College guidelines to report colorectal cancer resections and comparison with previous results? (2) To compare the frequencies of important prognostic factors to those of national standards.

Standards and Targets: (A) All the colorectal cancer resection reports should follow the minimum dataset by RCPath (MDS). (B) The frequency of important prognostic factors should be, mean number of lymph nodes examined at least 12, extramural venous invasion (EMVI) at least 25%, serosal involvement at least 20% for colonic cancers and 10% for rectal cancers.

Methodology: Retrospective done from Jan 2013-Dec 2013 (comparison with previous 5 years audit cycle). All cases of colorectal cancer resection included.

Results: (A) Compliance to Royal College minimum dataset. Clinical details 100%. Macroscopic description: Tumour site,size, distance to nearer cut end, relation to peritoneal reflection, plane of surgical excision 100%, tumour perforation 98%, and distance of tumour to dentate line in APR specimens 80%.

Microscopic Description: Tumour type, differentiation, margin involvement, extent of local invasion, number of lymph nodes 100%, extramural venous invasion 99%, distance of tumour to NPRM 97%, response to neoadjuvant therapy 97%, histologically confirmed distant metastasis 94%, background abnormalities 94% and maximum distance beyond muscularispropria 92%. Pathological staging: complete resection at all margins, Dukes stage, pT 100%, pN 98% and pM 85%.

(B) Frequency of prognostic indicators. Mean number of lymph nodes 24, frequency of EMVI 39%, frequency of serosal involvement in colonic cancers 34% and those of rectal cancers 12%.

Conclusion: In colorectal cancer, high-quality pathology reporting is vital. In general, all the important diagnostic criteria were included in the reports. 22 out of 24 core items were documented in above 90% of the cases. Our reporting frequency has been consisitenly high above standard over 5 years

P87

Assessment of Lymph Node Yield in Colorectal Cancer

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Purpose of the Study: The presence of lymph node metastasis is an important prognostic factor in colorectal cancer. Lymph node yield is a key parameter in the assessment of the quality of histopathology reporting of colorectal cancer excision specimens. The Royal College of Pathologists have set a standard of 12 for the median number of lymph nodes examined per specimen.

Methods: This study assesses the trend in lymph node yield from colorectal cancer excision specimens in a prospectively collected dataset compiled from the histopathology reports of 2646 patients who underwent surgery for primary colorectal cancer between 2005 and 2014 in a single institution.

Results: There was a significant increase in the total number of lymph nodes over the time period of this study from a mean of 14.91(median 14) in 2005 to a mean of 22.13 (median 21) in 2014, when all cases were assessed (n=2646). At least 12 lymph nodes were examined in 85% of cases. Over the same time period, in those cases where there had been no neoadjuvant therapy (n=2120), the mean number of lymph nodes examined rose from 15.36 (median 14) in 2005 to 22.44 (median 21) in 2014. In Dukes A cases the mean lymph node yield was 16.04 while Dukes' B cancers had a mean lymph node yield 19.48 and Dukes C cases had a mean lymph node yield of 18.42. Comparison of tumour site showed that proximal colon cancers had a mean lymph node yield of 19.46, distal colon cancers had a mean lymph node yield of 17.01 while rectal cancers had a mean lymph node yield of 19.6. The mean lymph node yield in cases following neoadjuvant therapy (n=526) increased from 12.03 in 2005 to 20.92 in 2014.

Conclusions: The data indicates that in this centre there is increasing lymph node yield from colorectal cancer excision specimens. The median number of lymph nodes examined surpasses the standard indicated by the Royal College of Pathologists.

P88

The Relationship of Lymph Node Yield and Proportion of Dukes' C Colorectal Cancers

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Purpose of the Study: The presence of lymph node metastasis is an important prognostic factor in colorectal cancer. The Royal College of Pathologists' guidelines on reporting colorectal cancer indicates that all lymph nodes should be examined as it is expected that an increase in the number of nodes examined would result in an increase in the number of lymph node positive (Dukes' C) colorectal cancers.

Methods: This study compares the lymph node yield from colorectal cancer excision specimens and the proportion of lymph node positive cases over time in a prospectively collected dataset compiled from the histopathology reports of patients who underwent surgery for primary colorectal cancer between 2005 and 2014 (n=2120). Patients who had received neoadjuvant therapy were excluded from the study.

Results: There was a significant increase over time in the total lymph node yield per case, from a mean of 15.36 in 2005 to a mean of 22.44 in 2014 (Pearson correlation, r=0.939, p<0.001). No such trend was observed in the number of Dukes' C cases, with 44.3% of cases in 2005 lymph node positive and 43.2% of cases lymph node positive in 2014 (Pearson correlation, r=-0.268, p=0.454). When bowel screen detected cases are excluded the mean lymph node yield increased from 15.48 in 2005 to 22.9 in 2014 (Pearson correlation, r=0.940, p<0.001). There was no increase in the number of Dukes' C cancers, with 45.7% of cases in 2005 lymph node positive and 44.2% of cases lymph node positive in 2014 (Pearson correlation r=-0.188, p=0.604).

Conclusions: The data indicates that in this centre, there is an increasing yield of lymph nodes from colorectal cancer excision specimens, without necessarily a similar increase in the number of lymph node positive colorectal cancers even when the confounding effects of bowel screening and neoadjuvant therapy are excluded.

P89
Audit of the Clinical Information Provided in Colorectal Histology Request Forms

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Background: By its very nature surgical pathology depends heavily on the input of clinicians and surgeons. The pathologist's need for adequate clinical information before diagnosis can be made has been highlighted in the past.

Aim: To audit the quality of clinical information provided in colorectal resection histology requests for the past 5 years. According to the Royal College of Pathologists' guidelines for reporting colorectal cancer histopatholy reports the following characteristics were examined: (1) the presence of a diagram of the surgical procedure, (2) if the cancer has been detected as part of the bowel cancer screening programme, (3) the histological type of tumour if known, (4) if there is history of inflammatory bowel disease or familial cancer, (5) the pre-operative stage of tumor, (6) whether or not pre-operative therapy has been given, when it finished and its nature, (7) if open, laparoscopic or robotic surgery has been performed, the type and dissection plane of the operation.

Methods: Data from 500 patients with large bowel resections were collected between 2/12/2009 and 18/11/2014.

Results: Out of the 498 histology request forms, only two had a diagram present (0.4%) and three (0.6%) reported that the tumour was detected in the bowel cancer screening programme. The histological type was reported in 72 out of 496 samples (14.52%) and the presence of IBD or familial cancer was reported in 2 out of 497(0.4%). The pre-operative stage of tumour was recorded in 27 out of 496 reports (5.4%) and the pre-operative therapy given in 56 out of 496(11.3%). Finally, the type of surgery and dissection were adequately documented in 221 out of 497 reports (44.5%).

Conclusions: Overall, the quality of reporting of clinical information in histology reports remains suboptimal. This audit identified important areas in which reporting quality needs to be improved.

P90
An Audit of Duodenal Biopsies Submitted for Histopathological Assessment of Possible Coeliac Disease

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Purpose of the Study: We performed an audit to assess local compliance with guidelines published by the British Society of Gastroenterology in 2010 and 2014 that recommend a minimum of four duodenal biopsies for the histopathological assessment of possible coeliac disease. The 2014 guidelines also recommend biopsies from the duodenal bulb (D1).

Methods: The records of 2744 adult duodenal biopsies, received for histopathological assessment of possible coeliac disease between December 2013 and November 2014 were reviewed.

Summary of Results: Fewer than the recommended minimum number of biopsies were taken in 58% of cases. The precise sample site was unclear in 51%. The diagnosis was 'normal' in 84% of biopsies and supportive of coeliac disease in 5%. D1 was sampled in 93 (3%) cases, with D1 the only site sampled in 22 (<1%). When D1 was sampled with D2-D4 biopsies, the same diagnosis was made in each biopsy pair in 53% of cases (38/71), while a different diagnosis was made in the D1 biopsies in 37% (26/71) (e.g. gastric heterotopia/metaplasia in 43%). In 10% of cases (7/71) the biopsies were reported together as one diagnosis. In no cases were D1 biopsies consistent with coeliac disease when accompanying D2 biopsies were normal. In three cases, features consistent with coeliac disease were present in the D2 biopsies but not the accompanying D1 biopsies.

Conclusions: Guidelines for adequate duodenal sampling are not always adhered to locally and the precise biopsy site is not always clearly stated. Endoscopic sampling of at least four biopsies, in line with existing and updated national guidelines, plus clear labelling of the site to aid accurate histopathological assessment of biopsies from different areas of the duodenum, is recommended. D1 sampling is rare in our institution; however, it has only recently been recommended in the updated 2014 guidelines, so this practice may increase in the future.

P91
Does the Bowel Cancer Screening Program Detect Significant Upper Gastrointestinal Pathology?

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Purpose of Study: To determine whether Bowel Cancer Screening Programme (BCSP) patients with non-malignant lower gastrointestinal (LGI) pathology have subsequent upper gastrointestinal (UGI) pathology which may account for a positive faecal occult blood (FOB) test.

Methods: LGI specimens from 333 patients were submitted for histological examination as part of the local BCSP between May 2008 and April 2010. From the same patient cohort, subsequent gastrointestinal (GI) biopsy results from the following 3-5 years were recorded retrospectively and analysed, with particular focus on UGI pathology.

Summary of Results: 332 patients were included (233 male, 99 female), with median age of 66 years (range 60-78 years). 601 specimen results were reviewed, with most patients having more than 1 biopsy. Initial BCSP specimens included 50 adenocarcinomas (15% of patients), 411 adenomas (257 tubular, 145 tubulovillous and 9 villous) with a high grade dysplasia (HGD) rate of 5.1%, 136 hyperplastic polyps, 23 inflammatory changes, 26 other findings and 46 normal. Subsequent LGI biopsies from the same patient cohort contained 261 adenomas with a HGD rate of 3.8%. 28 of the initial 322 patients (who had non-malignant LGI results) had subsequent UGI biopsies (including oesophageal, gastric and duodenal). These UGI results included 2 adenocarcinomas, 2 H.pylori change, 1 coeliac disease, 3 chemical gastropathy and 20 normal/minor inflammation.

Conclusion: A high rate of colorectal adenocarcinoma and adenomas are detected through the BCSP. A very small number of patients (2 out of 332) also had UGI malignancy following a diagnosis of LGI adenomatous polyps. Both presented with widespread metastatic disease more than 6 months after initial BCSP investigation. These cases seem to be exceptional in the context of the BCSP, which is not designed to detect UGI pathology.

P92
The Role of Ki67 Expression on Cellular Organisation Leading to Progression Towards Oesophageal Adenocarcinoma

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Introduction: Ki67 is a proliferation marker that is exclusively present in dividing cells and absent in resting cells. Its expression has already been studied in different cancers and used to understand the cellular organisation of Barrett's epithelium (Lavery 2014). However, very little is known about the cellular organisation based on Ki67 expression patterns in upper GI sequence. This study aims to examine the cellular organisation as defined by Ki67 expression patterns in upper GI cancer sequence.

Methods: Ki67 expression within Barrett's crypts was assessed in 40 cases (NBDE 11, LGD 15, HGD 14). The Barrett's crypts were divided into three equal regions: crypt base (bottom third), middle region and the surface (upper third), respectively. Ki67 was scored using the Allred system and analysed using one-way ANOVA with Bonferroni post-hoc analysis.

Results: One-way ANOVA showed significant difference across the three groups (p < 0.0001). Bonferroni post-hoc analysis showed significant difference in the surface architecture between NBDE and HGD (p < 0.0001) and LGD and HGD (p < 0.0001). For the middle region, although there was no statistical significance between the groups, NBDE and LGD and LGD and HGD showed statistical trends (p = 0.079 and p = 0.089 respectively). For the basal compartment there was significant difference between NBDE and LGD (p = 0.035).

Conclusion: This study showed for the first time a significant difference in the Ki67 expression between NBDE, LGD and HGD in the basal and surface regions. Middle compartments showed trends but additional NDBE, LGD and HGD groups need to be analysed to increase the statistical power. The results warrant further molecular analysis between the various groups and show a clear role for proliferation in the maintenance of the cellular architecture and organisation across the upper GI groups which might help in the understanding of the origin and development of OAC.

P93

Audit of Compliance with Standards in the Royal College of Pathologists Dataset for Colorectal Cancer (July 2014, 3rd Edition)

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Purpose of the Study: Identify whether the department is compliant with the following national standards set by the Royal College of Pathologists in their revised dataset for colorectal cancer histopathology reports (July 2014, 3rd edition):
• Median number of lymph nodes examined should be greater than twelve.
• Frequency of serosal involvement should be at least 20% for colonic cancers and 10% for rectal cancers.
• Frequency of venous invasion should be at least 30%.
• 95% of reports must contain structured data.
• 80% of cases are authorised within seven calendar days of procedure, 90% within ten calendar days.
Method: A retrospective audit reviewing all colorectal adenocarcinoma cases seen in 2013 (identified by a laboratory computer system search).
Summary of Results:
Total of 139 resection cases identified (104 colonic and 35 rectal).
• Median number of lymph nodes examined is 20
• Frequency of serosal involvement is 23% for colonic cancers and 2.9% for rectal cancers.
• Frequency of venous invasion is 30.9%
• 100% of reports contain structured data
• 16.5% of cases were reported within seven calendar days, 66.2% within ten calendar days
Conclusions: The department is compliant with most standards, except:
• Frequency of serosal involvement in rectal cancers (suggested contributing factors for this include effect of pre-operative therapy, tumour regression and recent changes in surgical practice).
• Turnaround times: suggested contributing factors include increased departmental workload, retirements and reduced reporting capacity.
The following action plan was implemented to improve compliance with standards:
• Ensure all pathologists are aware of results via presentation/dissemination of audit report
• Identify issues affecting turn-around times and improvement strategies
• Support recruitment to increase reporting capacity.
• Maintain awareness of the need to recognise serosal involvement in rectal excisions.
• Re-audit in 1 year.

P94

The role of IL-33/ST2 axis in Colorectal Cancer

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Background: ST2 and its ligand IL-33 are members of the TLR /IL-1 receptor family. Three isoforms of ST2 exist: a trans-membrane receptor (ST2L), a secreted soluble form (sST2), and a variant form (ST2V). Changes in IL-33 and ST2 have been reported in numerous cancers but their role in colorectal cancer (CRC) is unclear.
Purpose: To investigate the role of the IL-33/ST2 pathway in CRC.
Methods: ST2L and IL-33 mRNA levels were examined by qRT-PCR. sST2 and IL-33 serum levels were examined by ELISA. IL-33, ST2V and ST2L, were characterized by immunohistochemistry (IHC) on formalin-fixed paraffin-embedded CRC blocks.
Results: Expression levels of IL-33 and ST2L were examined in a cohort of 25 CRC cases. Levels of ST2L mRNA and protein were significantly reduced in CRC in comparison to adjacent non-tumour colonic mucosa as assessed by both qRT-PCR and IHC. Serum levels of IL-33 were also significantly reduced in this cohort in comparison to 15 healthy volunteers. These results were confirmed in a second patient cohort of 68 CRC cases analysed by IHC. ST2L showed decreased incidence and staining intensity with increasing TNM and pT stage. Neither ST2L nor IL-33 were over-expressed in colorectal metastasis relative to primary CRC. Following three year post-surgery analysis of cohort 2, cases with strong/moderate ST2L were associated with improved patients' survival in comparison to weak/negative cases. ST2V expression was unaltered in CRC relative to normal colon.
Conclusions: This data indicates a potential protective role for IL-33/ST2 in CRC. This research has been awarded the small grant scheme by the Pathological Society of Great Britain & Ireland.

P95

A Pilot Online Digital Archive of Colorectal Polyps and Teaching Cases for the Irish National Bowel Screening Programme

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Aim: The design and maintenance of a pilot online digital archive of archetype colorectal polyps and gastrointestinal (GI) teaching cases for the National Bowl Screening programme (BowelScreen) in the Republic of Ireland.
Methods: Suitable internal and referral cases were identified by BowelScreen consultants at Saint Vincent's University Hospital. These cases were subject to both internal and external review, by the Mater Misericordiae University Hospital, and represented typical examples of lesions seen in a National Bowel Cancer screening programmes (e.g adenomas, SSLs, adenomas with misplacement, TSAs). Representative slides, including immunohistochemistry, were anonymised and digitised using the Hamamatsu NanoZoomer Digital Pathology (NDP) whole slide scanner platform and associated software packages (NDP scan and view).
Whole slide images (WSI) were uploaded to secure cloud storage using a generic file transfer protocol program. WSI were collated into 18 cases and accessible via the PathXL gateway (pathXL.co.uk) by approved users via an online case referral and reporting system. Users were notified of pending cases via email and the viewing of WSI occurred within the user's web browser utilising an online version of NDP view program and did not require local use of propriety software. Each case was referred across the two participating sites and scored in four areas; diagnosis concordance, quality of WSI, web interface and the online referral and reporting system.
Conclusion: With the maturation of technology involved in digital microscopy a digital archive program is now a feasible approach to the standardisation of diagnosis and a useful adjunct to traditional optical microscopy in education within the National Bowel Screening programme.

P96

Diffuse Ganglioneuromatosis of the Gut: A Rare Case Report

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Introduction: Intestinal ganglioneuromatosis is a rare pathological condition of the enteric nervous system and involves proliferation of ganglion cells, Schwann cells and nerve fibres in the bowel wall Diffuse ganglioneuromatosis is rare in adults.
Case presentation: We are presenting an extremely rare case in a 48 years male with history of neurofibromatosis presenting with gripping abdominal pain. On colonoscopy, there were multiple polyps in the intestine with abnormal proximal ileum. On macroscopic examination, the bowel showed various nodular lesions measuring 1cm. On microscopy, there was transmural infiltration of diffuse spindle cells with fibromyxoid areas and had ganglion cells. The tumour cells were positive for S100, and focally for CD34. Alk and CD1a were negative. There were no features of malignancy.
Conclusion: In adult patients presenting with obstructive gastrointestinal symptoms, the rare possibility of ganglioneuromatosis should be considered and investigated in order to avoid unnecessary and extensive surgical intervention.

P97

A Trainee's Perspective of the Biomedical Scientist (BMS) Histopathology Reporting Pilot

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Purpose of the Study: To see if it is feasible to train BMS staff to undertake independent reporting and cut-up of gastrointestinal and gynaecological histopathology cases.
Methods: This three year training programme commenced in 2012 and uses the training of junior medical staff in histopathology as a template. A portfolio must be completed each year. The minimum components of the portfolio are evidence of cases dissected and reported, eighteen workplace based assessments, an audit and an educational case report. Trainees must pass an Objective Structured Pathology Examination (OSPE) at the end of Year One. Year Two is entirely portfolio based. Trainees must sit an Exit Examination at the end of Year Three.
Summary of Results: Five candidates from the first intake sat and passed the OSPE in 2013. The same five candidates are currently in Year Three and will sit the Exit Examination in September 2015.
Conclusions: A committed and conscientious BMS can learn how to report histopathology cases. However, if this is to be achieved, the department in which he or she works must also be committed and supportive.

P98

This abstract is not available before the meeting

P99

Carcinoid Tumour of the Appendix: A Case Report

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A case of a 24 years male operated on for acute appendicitis and an incidental finding of a carcinoid tumour at the tip is reported. The tumour was less than 2 cm in greatest dimension but it infiltrates through the wall of the appendix into the surrounding fat. It stains positive for the neuroendocrine markers. Carcinoid tumour of the appendix is unusual, but it has to be looked for during examination of appendectomy specimens done for appendicitis (0.5%). Women are more frequently affected than men (3:1) and the tumour is usually small less than 1 cm in diameter and frequently located at the tip. It is usually diagnosed incidentally after an operation for acute appendicitis and sometimes during other procedures (colectomy, cholecystectomy and others). The tumour rarely metastasis to the liver and this is usually related to the tumour diameter) and can cause a "carcinoid syndrome": flush, diarrhea bronchoconstriction, cardiac valve disease. Diagnosis is made by the pathologist and staging by conventional radiologic procedures (TAC, US), dosage of neuroendocrine mediators such as 24 hours urinary 5-HIAA. Simple appendectomy is adequate treatment for appendicular carcinoids less than 1 cm in diameter. Adequate treatment for tumours greater than 2 cm is right hemicolectomy. The mangement of tumours 1 to 2 cm range is controverisal, but generally, appendectomy alone is sufficient except when meso-appendix is invaded. Carcinoid tumour of the appendix has a good prognosis with a 5-year-survival rate, of 85-100%.

P100

The Prevalence of Epithelial Changes in Helicobacter Pylori-Associated Gastritis in Oman: A Retrospective Study

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There is Strong association between H. pylori gastric infection and epithelial changes and progression to cancer. It has been shown that H pylori infection is strongly associated with high proliferative activity and it could be a risk of initial step of gastric carcinogenesis. The aim of this study was to examine the association between epithelial changes in the gastric mucosa and gastric H pylori infection in Oman by retrospective examination of the gastric biopsies for patients presented to Sultan Qaboos University hospital (SQUH) in 2013. A total of 697 biopsies were studied with a prevalence of H pylori infection in 34% with about 13% showing epithelial changes, mainly intestinal metaplasia in 10% out of the H pylori positive cases, a few cases with low grade dysplasia and reactive atypia. In cocnlusion intestinal metaplasia was the main epithelial change that was related to H pylori infection. Further studues are required to investigate the relation between H pylori infection and the progression to gastric carcinoma.

P101

The Effect of Changing Surgical Technique on Oncological Outcomes for Abdomino-Perineal Excision of Rectum in a DGH Setting

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Purpose of the study: Low rectal carcinoma may require abdomino-perineal excision of the rectum (APER), which has been associated with higher rates of tumour perforation and circumferential margin (CRM) involvement than anterior resection. This increases the risk of local recurrence and may necessitate adjuvant treatment. The Extralevator Abdominoperineal Excision of Rectum (eLAPE) in the prone position has been found to improve these outcomes and has been encouraged by the Low Rectal Cancer National Development Programme (LOREC). We aimed to assess the effect of increasing the practice of eLAPE on the histological and oncological outcomes in these cases in the Mid-Yorkshire NHS trust, a large District General Hospital.

Methods: In 2011 the number of surgeons routinely performing APER was reduced and all those performing the procedure had been trained in the cylindrical resection technique. Joint operating and laparoscopic procedures were encouraged. A retrospective review of case notes and histological reports between 2009 and 2012 was performed (before and after sub-specialisation). Patient demographics, histological findings and complications including local recurrence were recorded.

Summary of Results: Between 2009 and 2011, 39 APERs were performed, with tumour perforation in 5 (13%) and CRM involvement in 8 (21%) of cases. After sub-specialisation, 28 were performed. None were perforated and 2 cases (7%) showed margin involvement. Local recurrence occurred in two cases before specialisation and none after 2011 at the time of follow-up. Joint operating and subspecialisation increased the number of cases performed by each surgeon, and the number performed laparoscopically.

Conclusions: eLAPE in conjunction with departmental restructuring significantly improves immediate oncological outcomes in a DGH setting, with no effect on 30 day mortality. The technique may reduce local recurrence, although longer follow-up would be required.

P102

Systems Biology Approaches to Cervical Pre-Cancer Diagnostics

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Background: Systems biology uses computational and simulation approaches to interrogate gene expression datasets and explore biological pathways. By employing systems biology and data mining tools we can identify new biomarkers. Our objective was to ascertain the utility of a novel panel of systems biology derived biomarkers in cervical pre-cancer for more accurate grading and stratification of CIN disease.

Methods: This project is conducted within the framework of an FP7 funded programme "SYSTEMCERV". Gene pathways were analysed using MATLAB and SIRENE. Along with accessing KEGGS online database for gene prediction and DAVID for gene functional classification, we identified a novel panel of biomarkers. Gephi software was used to visualise communities of genes related to cervical pre-cancer and cancer progression. Clinical validation was performed by immunohistochemistry on a range of cervical LLETZ specimens (Normal, CIN1, CIN2 and CIN3). All patients gave written informed consent. In parallel, p16 IHC was performed on all specimens as a benchmark stain.

Result: The biomarker panel included TP63, epiregulin and desmoglein-3. Biomarker expression patterns were evaluated on 113 clinical samples, normal [n=13], CIN1 [n=32], CIN2 [n=34] and CIN3 [n=31]. Altered expression patterns were identified in CIN lesions as compared to normal cases. Desmoglein-3 showed significant difference in expression across different grades of CIN. Epiregulin played a suggestive role in identifying virally infected cells. TP63 showed the strongest correlation to p16. Expression increased with disease progression, indicating the capability of TP63 to demarcate between CIN 1, 2 and 3.

Conclusion: Novel biomarkers have the potential to distinguish between different grades of CIN based on protein expression status. This systems biology-based approach for identifying novel markers within gene pathways may significantly improve grading of CIN.

P103

Defining the Raman Spectroscopic Signature Associated with Low and High Grade Cervical Disease

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The mortality associated with cervical cancer can be reduced if the disease is detected at the early stages of development or at the pre-malignant stage. The Pap smear is the current screening method, but is highly subjective and can often exhibit low specificity and sensitivity. For this reason, either a replacement or supportive technique is necessary to improve the quality of cervical cancer screening. Raman spectroscopy is a powerful tool that can generate a biochemical fingerprint of a sample in a rapid and non-destructive manner.

In this study, Raman spectroscopy has been applied to the investigation of cervical cells from PreservCyt specimens. Raman measurements were taken from the nuclei of cervical cells from normal, CIN1, and CIN3 samples. These spectra were processed, analysed and used to define a spectral signature for each grade of cervical disease. Principal Component Analysis (PCA) was used to discriminate between the two data sets.

Distinct Raman spectral differences were detected between normal, CIN1 and CIN3 cells. Notably, it was possible to observe spectral peak shifts representing fluctuations in Guanine (DNA/RNA), CH deformation in proteins and carbohydrates, Carbon-carbon double bonds in Phenylalanine, Tyrosine and Tryptophan, and Amide I. The PCA showed an excellent discrimination between the data sets.

This study has shown that Raman spectroscopy can detect subtle changes between cervical cells, and may be a powerful tool for improved diagnosis of cervical dysplasia.

P104

The Role of the TLR4 Pathway and the Spindle Assembly Checkpoint in Ovarian Cancer Prognosis

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Background: MyD88 and MAD2 are two potential prognostic biomarkers that have been investigated in ovarian cancer. High MyD88 and Low MAD2 IHC staining is associated with reduced PFS, both markers are also linked to paclitaxel chemoresistance.

Objectives: The main objective of this study was to assess the *in vitro* relationship between MAD2 and MyD88, through alteration of MAD2, MyD88 or its receptor TLR4 in two ovarian cancer cell lines using siRNA targeting MAD2, TLR4 or MyD88 and a MyD88 overexpression plasmid vector. Following overexpression/siRNA knockdown procedures, MyD88, TLR4 and MAD2 expression was assessed through qPCR and Western Blot analysis. Mir-433, Mir-21 and Mir-146a gene expression was also assessed by qPCR. Furthermore the effect of TLR4/MyD88 knockdown on chemoresponse was assessed in SKOV-3 cells using the CCK-8 assay.

Results/Discussion: It was found that knockdown or overexpression of MyD88 in SKOV-3 or A2780 cells respectively or knockdown of TLR4 in SKOV-3 cells had no effect on MAD2 expression or the expression of Mir-21, Mir-433 and Mir-146a. Interestingly however knockdown of MAD2 in both cell lines induced a 3 fold increase in TLR4 expression, furthermore knockdown of TLR4 in SKOV-3 cells was shown to restore chemosensitivity to paclitaxel.

Conclusion: The results demonstrate a potential *in vitro* link between TLR4 and MAD2 and support a role for TLR4 in paclitaxel chemoresistance.

P105

The Complexity of Ovarian Cancer Resistance Mechanisms: A Novel, Clinically Relevant, *in-vitro* Investigation

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Ovarian cancer (OC) is the leading cause of death from a gynaecological malignancy. Standard chemotherapy often fails and patients relapse with chemoresistant disease. Novel carboplatin and taxol resistant cell lines were developed from UPN251 OC cells in a clinically relevant selection strategy to better understand resistant mechanisms in OC. UPN251-7C models carboplatin resistance and UPN251-7T models taxol resistance. UPN251-6CALT and UPN251-6TALT were exposed to alternating treatments of both agents during development. Affymetrix arrays were used to characterise gene/miRNA signatures linked with the development of chemoresistance in OC cell lines UPN251-7C and UPN251-7T. Bioconductor software, DAVID v6.7 and miRNA-target interactions (MTIs) analysis was carried out to identify de-regulated genes/miRNAs, gene pathways and gene/miRNA interactions involved in resistance. UPN251 sublines developed using taxol were significantly resistant to taxol, vinblastine and olaparib (P-gp substrates), and reversible with elacridar (P-gp inhibitor) treatment. Significant up-regulation ABCB1 was seen in UPN251-7T which was reflected at the protein level. SRPX2 was highly up-regulated in UPN251-7T. GLI3 and CCL20 were up/down-regulated respectively in UPN251-7C. GLI3 had a validated interaction with miR-205 down-regulated in UPN251-7C. LIN28B was highly deregulated in UPN251-7C and UPN251-7T and had a validated interaction with let-7i, down-regulated in UPN251-7C. P-gp over-expression is a dominant mechanism for taxol resistance in our cell lines. Mechanisms for carboplatin resistance are more complicated. The top deregulated genes are involved in numerous pathways including apoptosis, cellular transformation, signal transduction, and cell migration. Bioinformatics analysis and literature review identify LIN28B, GLI3, CCL20 and SRPX2 and miRNAs let-7i and miR-205 as strong potential biomarkers for carboplatin/taxol resistance in OC.

P106

Cervical Glandular Neoplasia: The Influence of Excision Procedure on Margin Status

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The incidence of cervical glandular lesions is increasing. As these lesions frequently occur in women of reproductive age, the choice of treatment can impact on future fertility. Although cold knife cones (CKC) have been traditionally advocated for treatment of adenocarcinoma in situ (AIS), large loop excisions of the transformation zone (LLETZ) are increasingly used. We analysed excisions from 111 patients where AIS was confirmed prior to procedure over 5 years to assess the influence of excision procedure on final margin status. We tabulated whether margins were involved, close (lesional tissue less than 5mm from margin) or excised.
Results: LLETZs were performed in 70% (78 of 111 patients), CKC excision in 30% (33 of 111). Women who had LLETZs were younger than those having CKC excision (32.8 years versus 35.4 years). Positive margins were present in 21% (16 of 78) and close margins in 27% (21 of 78) LLETZ cases. For CKC, margins were positive in 9% of cases (3 of 33) and close in 6% (2 of 33). 28% of patients who had an initial LLETZ required a second procedure (11 patients had a further LLETZ while 11 had CKC excision).
Conclusion: Although complete excision is more frequently observed when CKC is performed, compared to LLETZ, for the treatment of AIS, CKC can impact on future pregnancies. Any cervical intervention must be cognisant of the sometimes conflicting needs of cervical preservation and the need for excision of a pre-malignant lesion.

P107

The Role of MAD2 and MyD88 as Prognostic Indicators in Ovarian Cancer

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Background: The prognosis of epithelial ovarian cancer is poor in part due to the high frequency of chemoresistance. Recent evidence points to the Toll-like receptor-4 (TLR4), and particularly its adaptor protein MyD88, as one potential mediator of this resistance. Downregulation of MAD2, a key component of the spindle assembly checkpoint complex, has also been linked with paclitaxel resistance . Both markers have individually been shown to be associated with poor outcome in ovarian cancer. High MyD88 and low MAD2 immunohistochemical staining is associated with reduced progression free survival. The main objective of this study was to assess the combined utility of MAD2 and MyD88 in predicting patient prognosis.
Methods: Two tissue microarrays composed of cores from 51 high grade serous epithelial ovarian cancers patients were constructed and stained for MAD2 and MyD88. Staining was scored based on previously derived scoring schemes for MyD88 or MAD2 staining. The mean overall score from triplicate cores was then used to classify patients into high and low staining categories.
Results: A trend towards reduced progression free and overall survival was observed in patients with high myd88 and low mad2 expression.
Conclusion: The results demonstrate the combined utility of MAD2 and MyD88 as predictors of prognosis in ovarian cancer.

P108

Identification of the Chemoresistant Component of a Novel Ovarian Cancer Stem Cell Hierarchy

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Purpose: Ovarian cancer is characterised by high rates of terminal, chemoresistant recurrence. Although chemoresistance is known to be a property of Cancer Stem Cells (CSCs), the mechanism is poorly understood. We have previously identified a novel four-member CSC Stem-Progenitor cell hierarchy for ovarian cancer. The aim of this study was to characterise the contribution of each member of the ovarian CSC hierarchy to chemoresistance.
Methods: The CSC hierarchy was assessed for tolerance to chemotherapy drug cisplatin (MTT assay) both as components of the parent population (A2780 cell line) and as isolated cell types. The hierarchy was additionally assessed in the long-term cisplatin-adapted 'A2780cis' cell line. Cell types were analysed and isolated via flow cytometry and assessed for stem cell characteristic via single-cell asymmetric division and murine xenograft tumourigenicity assays, and molecularly characterised (whole transcriptome arrays).
Results: Cisplain dose-response assays from A2780-derived CSC sub-populations indicated that only one of the four populations within the hierarchy had a high cisplatin-tolerance (IC50=10uM) compared to the other populations (IC50=4uM). This was notable as the relative cisplatin IC50s for the A2780 and A2780cis parent cell lines are 4uM and 11uM respectively. Treatment of the parent A2780 cell line with the IC50 (48 hours) resulted in a proportional 50% loss in each of the four cell types, suggesting that this specific CSC sub-population adapts to cisplatin over a longer period of time.
Conclusion: Although CSCs are known to be chemoresistant, the mechanism though which this is achieved is poorly understood. Our data indicates that only some members of a CSC hierarchy are responsible for chemoresistance. Notably, this sub-population appears to possess inherent chemoresistance in pre-treatmentcells. As such, it should be possible to target these CSCs in the primary malignancy to prevent chemoresistant recurrence.

P109

Mixed Sex Cord Stromal Tumour of Ovary Containing Pseudoendometrioid Sertoli-Leydig Cell Tumour: a Rare Case Report

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Mixed sex cord-stromal tumours of the ovary are very rare. We report a case of mixed sex cord -stromal tumour (also referred to as gynandroblastoma) containing both Sertoli-Leydig cell tumour and adult granulosa cell tumour in a female 61 years old who presented with postmenopausal bleeding. On histology, the majority of the tumour represented an unsual form of well differentiated Sertoli-Leydig cell tumour with a pseudoendometrioid appearance. Minor foci of classic adult granulosa cell tumour were present. On immunohistochemistry, the tumour was diffusely positive for inhibin and SF1 and focally for calretinin, ER and CD56. EMA, PAX8 and CK7 were negative. As far as we are aware, this is the first report of an ovarian mixed sex cord-stromal tumour containing a component of pseudoendometrioid Sertoli-Leydig cell tumour.

P110

Hypertrophic Herpes Simplex Simulating Vulval Cancer in an HIV Positive Patient: Case Report and Literature Review

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A 37 year old female patient presented with bilateral painful warty lesions on the labia majora. The patient had had HIV for a long time and was on highly active antiretroviral therapy. She also suffered chronic renal failure requiring haemodialysis three times weekly. Clinically, the lesions were highly suspicious of vulval cancer. The lesions increased significantly in size over a short period of time (2 months) requiring surgical resection under general anaesthesia.

Histological examinations revealed polypoid lesions with prominent pseudoepitheliomatous hyperplasia and dense inflammatory infiltrate, composed mainly of lymphocytes and plasma cells, extending to the hypodermis. Numerous abscesses with large numbers of eosinophils were present withinin the hyperplastic epithelium. The typical intranuclear inclusions of herpes simplex virus (HSV) were identified. HSV immunohistochemistry was positive.

This is a rare case of vulval HSV warts mimicking cancer. Oral acyclovir was administered following surgery and resulted in good control. Literature review shows only 6 previously described cases of verrucous HSV, Types 1 and 2, simulating neoplasia in patients with AIDS on antiretroviral therapy.

P111

Uterine Angioleiomyoma – A Case Report and Literature Review of a Rare Variant of Uterine Leiomyoma

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Angioleiomyoma is a pathological entity which is typically encountered in the subcutaneous tissue of the lower extremities, but very rarely, cases are reported in the uterine corpus. In this location, they are viewed as a variant of conventional uterine leiomyoma, and their characteristic histological appearance can result in diagnostic confusion. We report a case of uterine angioleiomyoma from a 51 year old lady who presented with menorrhagia and a fibroid uterus.

A key feature of uterine angioleiomyoma is the juxtaposition of intersecting fascicles of smooth muscle cells with abundant thick walled arteriolar-type blood vessels. The differential diagnosis includes conventional leiomyoma, angiomyofibroblastoma, endometrial stromal nodule and PEComa.

We present the clinical, histological, and immunohistochemical findings of this rare tumour variant. In addition, we provide a literature review encompassing the 16 previously reported cases, and summarize key points helpful in distinguishing this tumour from other histologically similar uterine lesions.

P112

The Demographics of Uterine Cancers – A Teaching Hospital Perspective

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Purpose of the study: The demographics of endometrial cancer have evolved over recent years. These factors pose management dilemmas for women deciding to delay their fertility. We analyse premenopausal and postmenopausal women with endometrial cancer with a view to documenting the local aetiological and demographic factors that influence both the management and survival.

Method: We retrieved 280 sequential uterine cancers from our pathology database between July 2010 to June 2012. All relevant pathology, imaging and case notes were reviewed and the data analysed. The cut-off age for premenopausal to postmenopausal was taken as 52 years and the endometrial cancer subtypes(endometroid, serous, clear cell and carcinosarcoma) were documented for both groups together with history of other malignancies, recurrence and survival.

Summary of Results: In the premenopausal (PRM) group 77% had type I cancers and 23% had type II cancers. In the Postmenopausal(PM) age group 55% had type I cancers and 45% were type II. 77% had stage 1A disease in PRB vs. 17% in PM. Recurrence was 18% for type I and 20% type II for PRM vs. 9% for type I and 40% for type II in PM. 9% of PRM women had carcinosarcoma vs. 8% in PM group. No PRM patients with carcinosarcoma had a previous diagnosis of other malignancies however 29% of PM group with carcinosarcoma had breast cancer of which 100% were taking tamoxifen.

Conclusions: The two major subgroups showed a mark distinction in the incidence of the type I and type II cancers. Furthermore, we found 29% with carcinosarcoma in the postmenopausal group had a previous history of breast cancer. When these patients were matched to age and stage matched controls the survival was poorer.

P113

Should We Follow-Up Endometrial Polyps? We Present a Teaching Hospital's Experience

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Purpose of the Study: The follow-up of endometrial polyps poses a clinical dilemma; no guidelines currently exist. Risk factors are age, obesity, hypertension and tamoxifen use. Patients may be asymptomatic or present with abnormal uterine bleeding. The literature contains proposals for follow-up but none have been adopted. Risk of malignancy is considered low (0-12.9%). We audited current follow-up practice within a teaching hospital with histological outcome.

Methods: Retrospective analysis of 4 years of endometrial curettings from a Trust histopathology database identified 302 patients with polyps. Polyps with hyperplasia were followed-up over 12-16 years. Divided into pre- and post-menopausal groups.

Summary of Results: 47 of 302 patients with polyps had hyperplasia: 6 atypical, 28 complex, 12 simple and 1 unclassified. 1 polyp contained adenocarcinoma at presentation. Only 26 patients with hyperplasia were followed-up. 10 patients underwent hysterectomy within the follow-up period. Of 10 hysterectomy specimens; 2 had carcinoma, 6 had hyperplasia (2 with and 4 without atypia), 2 had no endometrial pathology. 16 of the 47 cases with hyperplasia followed-up with pipelle/curettage monitoring: 13 cases had no endometrial hyperplasia or neoplasia, 1 complex hyperplasia without atypia and 2 were inadequate. Of the 6 patients with atypical hyperplasia, 2 regressed within 3 years.

Conclusions: 2 cases progressed to carcinoma. One symptomatic and post-menopausal, the other had HNPCC. Progression time was 5-18 months. We are in agreement with literature stating regression can occur in hyperplasia. Follow-up of polyps with hyperplasia should be confined to symptomatic post-menopausal patients and those identified as high risk.

P114

Primary Mucinous Eccrine Adenocarcinoma of the Vulva – A Rare Malignant Adnexal Neoplasm at an Unusual Site

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Primary mucinous eccrine adenocarcinoma of the skin is a rare adnexal neoplasm, typically involving the head and neck region in the elderly population. Here we present a case of primary mucinous eccrine adenocarcinoma of the vulva; occurrence at this site is extremely rare, with only five cases published in English literature.

A 62 year old female presented with a 10mm vulval lesion, clinically suspected to be an inclusion cyst. The lesion was removed and sent for histopathological assessment. Histological examination revealed a well circumscribed, partly encapsulated tumour composed of rounded and irregular nests of polygonal epithelial cells with scattered lumina, suspended in pools of extracellular mucin. The epithelial cell nuclei displayed a uniform chromatin pattern with small distinct nucleoli. The mucin pools stained positive for Alcian blue and dPAS. Immunohistochemical staining demonstrated positivity for CEA, CK7, GCDFP, oestrogen receptor, progesterone receptor, synaptophysin and chromogranin. Immunostaining was negative for CK20, CDX2, CA-125, TTF-1, CH5/6, CK14, HER-2, WT-1, CD56 and S100. Ki-67 proliferation fraction was approximately 5%. Overall, the findings were those of a mucinous eccrine adenocarcinoma with neuroendocrine differentiation. Following multidisciplinary discussion, and negative imaging of the breasts and gastrointestinal tract, a diagnosis of primary mucinous eccrine adenocarcinoma of the vulva was reached.

Only a handful of cases of primary mucinous eccrine adenocarcinoma of the vulva have been reported. Metastatic disease, particularly from breast and colon, must be excluded. Follow up data from patients with primary mucinous eccrine adenocarcinoma of the skin suggests a high local recurrence rate (29.4%), necessitating close follow-up. However, risk of metastasis is low (9.6%).

P115

Recurrent, Multifocal Granular Cell Tumour of the Vulva

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We present the case of a 40 year old woman with multifocal granular cell tumours (GCTs) of the vulva with local recurrence despite treatment with surgical excision. On initial clinical presentation a painless vulval lesion was thought to represent a calcified sebaceous cyst but on histopathological examination this was found to be a GCT. The lesion recurred and at the time of re-excision a further lesion was identified which was also found to be a GCT.

Whilst multifocality and propensity for local recurrence at this site make treatment and follow-up challenging, their rarity and non-specific appearances make clinical diagnosis difficult. We discuss the clinico-pathological features of GCTs of the vulva with special attention paid to the issues surrounding multifocality.

P116

An Unusual Case of Extrauterine Endometrial Sarcoma with Deciduiod Features

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Introduction: Extra-uterine endometrial stromal sarcoma is very rare and therefore can pose diagnostic difficulty in both excision and biopsy material. However, it usually develops on a background of endometriosis and.

Case Report: A 79 year old woman presented with six months history of lower abdominal symptoms. She was anaemic and had elevated CA125. Computed tomography study showed a large multi-septated mixed solid and cystic mass (22 cm) arising out of the central pelvis and extended just above the umbilicus. The patient was treated by a hysterectomy, bilateral salpingo-ophorectomy and omentectomy. The histology showed endometriosis involving the serosa of the uterus and the adjacent tumour was composed of cells with abundant eosinophilic cytoplasm reminiscent of decidualised stroma. Adjacent to the tumour abundant reactive mesothelial cells, areas of necrosis and florid inflammation, including foamy histiocytes was also noted. A wide variety of immunohistochemical reactions were performed. Initially, the lesion was diagnosed as florid endometriosis with a deciduoid appearance. However, a large cystic mass in the abdomen recurred after 8 months. A CT-guided biopsy was performed and following review and second opinion of the first biopsy and recurrent tumour a diagnosis of endometrial stromal sarcoma arising in an extra-uterine location from endometriosis was made.

Conclusion: It is important to be aware that endometrial stromal tumours may have epithelioid/deciduoid features and the use of appropriate immunohistochemistry is helpful in reaching an accurate diagnosis.

P117

A Rare Case of Vulval Myxoid Chondrosarcoma

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Introduction: Primary Extraskeletal Myxoid Chondrosaroma (EMC) of the vulva is a rare mesenchymal neoplasm. The myxoid tumour differential diagnosis on a core biopsy can be quite challinging. To date, few cases have been reported in the literature.

Case Report: A 42-year old woman noticed a swelling on the right side of the labia, thought to be a Bartholin's cyst in 2011. She was managed conservatively. She had drainage and marsupialization under general anaesthesia. This resulted in extreme bruising of the vulva. This was managed with antibiotics and non-steroidal anti-inflammatory medication, and it resolved after 3 weeks. Six months later, the patient presented again with a persistent vulval mass. A biopsy was obtained under general anaesthesia, and it showed a myxoid tumour with differential diagnosis of low grade chondroid tumour. An MRI was performed to assess the extent of the disease. The tumour was excised. At surgery, a 7 x 5 cm lobulated, extremely vascular vulval tumour was found. The tumour was inseparable from the inferior pubic ramus of the pelvic bone. A complete macroscopic resection was obtained. Histology confirmed low grade myxoid chondrosarcoma.

Conculsion: Vulval lesions with unusual characteristics or insidious evolution in the labia majora or Bartholin's glands area should be carefully and promptly investigated. Differential diagnosis of myxoid tumours in the vulva should include myxoid chondrosarcoma amongst other diagnoses.

P118

An Investigation Into the Prevalence of Transformation Within a Cohort of Diffuse Large B Cell Lymphoma Patients

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Diffuse Large B Cell Lymphoma (DLBCL) is the most common high grade lymphoma in the UK. A proportion of DLBCL occur as the result transformation of a pre-existing low-grade lymphoma.

We have analysed a cohort of 265 patients diagnosed and treated in Bristol for DLBCL over a 5-year period. 47 patients were considered transformed from a pre/co-existing low-grade component. 31 (66.0%) patients had documented evidence of low-grade lymphoma in the previous or in the same biopsy. Of these 31 cases, 13 arose from Follicular Lymphoma, 13 from Marginal Zone Lymphoma, 3 from Chronic Lymphocytic Lymphoma, and 1 from Lymphoplasmacytic Lymphoma. In 16 (34.0%) patients, discordant low-grade lymphoma was identified in the bone marrow during staging investigations. 4 were classified as Follicular Lymphoma, 2 Marginal Zone Lymphoma, 2 Chronic Lymphocytic Lymphoma, 1 Lymphoplasmacytic Lymphoma, 1 Non CLL -like Monoclonal B-cell Lymphocytosis. Five had an accompanying a low-grade component that could not be classified. None of the transformed high-grade lymphoma cases were EBV positive.

In our cohort, we did not observe statistically significant difference in survival between the Transformed and Non-Transformed cases. An equal proportion of cases transformed from Follicular Lymphoma and Marginal Zone Lymphoma. 19.1% of Transformed patients had a previous history of another cancer, compared to 12.1% of Non-Transformed cases. Age, gender and a history of autoimmune disease were not associated with transformation. Transformed Chronic Lymphocytic Lymphoma, although rare, was as described highly aggressive. This study provides further information about nature of DLBCL with evidence of an associated low grade component.

P119

An Investigation Into the Presentation and Nature of Diffuse Large B Cell Lymphoma Within a Large Patient Cohort

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Diffuse Large B Cell Lymphoma (DLBCL) is a high-grade cancer, accounting for approximately one third of lymphoma cases in the UK. DLBCL is an umbrella term, encompassing multiple distinct disease entities.

We have studied a cohort of 265 DLBCL patients, diagnosed and treated in Bristol between 2009 and 2014. Clinical, epidemiological and histological data on each patient was complied into purpose-built database.

The age at presentation ranged from 23 to 96 years, with a mean age at 68 years. 121 patients were female and 144 male. Disease was nodal in 43.8% and extranodal 56.2%. The most common extranodal sites were the GI tract (11.3%), skin (6.8%), CNS (4.2%), bone (4.2%) and ocular (2.3%). All DLBCL subtypes were observed. DLBCL, NOS was the commonest (87.9%), T Cell/Histoiocyte-Rich Large B Cell Lymphoma (4.2%) was the next most common subtype, followed by Primary DLBCL of the CNS (3.0%). A modified R-IPI (the patient performance score was unknown) was used to stratify the patients into four risk groups and was found to be predictive of patient outcome. The average LDH level was 733.5 IU/L, well above 480, the upper limit of the normal range. Of the cohort of patients, 52.8% achieved remission, 15.5% were alive with disease at the end of the study and 31.7% are now deceased. The majority of patients that did die did so within a year of diagnosis. In addition, 27 Bcl-2 negative patients were identified, with a mean age at diagnosis of 66.8 years. 11 of these patients were female and 16 male. The mean LDH level was 747.5 IU/L. 51.9% of the Bcl-2 negative cases are in remission, 25.9% are alive with disease and 22.2% are deceased, none of these proportions are significantly different from the cohort as a whole.

P120

Composite Lymphoma of Diffuse Large B Cell Lymphoma with Hairy Cell Leukemia

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We have recently encountered an unusual case of composite lymphoma in a 63 year old gentleman. He presented with abdominal, retroperitoneal and mediastinal lymphadenopathy and enlarged spleen. Based on the core biopsy of a retroperitoneal lymph node, the diagnosis of diffuse large B cell lymphoma was made. A subsequent staging bone marrow however, revealed diffuse and heavy infiltration by hairy cell leukemia (HCL). Molecular genetic analysis of the bone marrow sample confirmed the BRAF c.1799T>A; p.Val600Glu (V600E) mutation commonly associated with HCL. Retrospective analysis of the retroperitoneal lymph node core biopsy revealed two populations of lymphoid cells. In addition to the dominant diffuse large cell component, there was a small population of small lymphoid cells showing hairy cell phenotype. The diffuse large B cell component was negative with hairy cell markers. Molecular analysis confirmed the BRAF mutation. Fluorescence in situ hybridisation (FISH) showed the presence of a BCL2 rearrangement in a proportion of cells. Therefore this case is best regarded as a composite lymphoma of diffuse large B cell lymphoma with hairy cell leukemia rather than blastic transformation of hairy cell leukemia. To the best of our knowledge, simultaneous occurrence of diffuse large B cell lymphoma and hairy cell leukemia in a lymph node has not yet been reported in the literature.

P121

Bone Marrow Aspirates and Trephine Biopsies: An Audit of the Effects of Integrated Haematopathology Reporting

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Bone marrow examination by aspirate and trephine biopsy is an important haematological investigation. Ideally, aspirate findings should inform examination of the trephine biopsy, but if the two modalities are separate the aspirate report can be delayed and histopathologists may assess the trephine biopsy without being aware of the aspirate findings. We audited the availability of aspirate results to the histopathologist examining trephine biopsies, over the period in which our department implemented an integrated haematopathology reporting system. The effects on diagnostic concordance, turnaround times and immunohistochemistry requesting were also assessed.

The setting was a regional specialist haematopathology centre. Prior to integration, a prospective audit of 101 consecutive trephine biopsies received by a senior haematopathologist was carried out, against standards set by the International Committee for Standardisation in Haematology. Data were collected from hospital computer systems. The move to integrated reporting involved the installation of new software (HiLIS) to specifically handle integrated haematopathology data. Ten months later, a retrospective analysis of a further 100 cases was performed using HiLIS data. Prior to integration, 35% of aspirates were reported within 3 days, and access to the aspirate report was available at time of examination for 61% of trephine biopsies. After integration, 96% of aspirates were reported within 3 days, and reports were available at time of examination for 100% of trephine biopsies. Diagnostic concordance was 72% initially, and 100% after integration. The mean number of immunostains requested per case was unchanged (5.9 vs 5.7).

Our findings show integrated reporting has markedly increased the availability of aspirate reports to the histopathologist, and improved diagnostic concordance. This new model benefits the haematologist, histopathologist and patient.

P122

The Value of Clonality in Lymphoma Diagnosis: Experience of a Tertiary Referral Molecular Laboratory

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Introduction: Clonality studies are carried out when the diagnosis of lymphoma is particularly challenging. The detection of clonality in lymphoproliferative lesions suspicious for lymphoma can be a valuable supplementary tool as it has a high positive predictive value. Clonal studies can also help to distinguish recurrent or residual disease from reactive inflammation. However false positive and negatives are common and can be attributable to several factors, including poor DNA quality.

Methods: 206 cases reported over a six month period (Jun-Nov 2014) were retrospectively reviewed, 62% of which were referral cases. We investigated various aspects of clonality studies; including DNA quality, fixation method, clinical information provided and correlation between the morphological/immunophenotypical findings and clonality results using Euroclonality/BIOMED-2 primers (IgH, IgK, TCR-B and gamma-delta).

Results: 166 (81%) had adequate DNA quality, 29 (14%) poor DNA and 11 (5%) had inadequate DNA quality. External cases had better DNA quality in the majority of cases. Clinical information was provided in 75% of local cases and 53% of external cases. In 56% of cases clonality results supported the initial histological report. 9 cases showed clonal expansion despite a benign process on histology. 21 suspected cases lymphoma (13 B-NHL and 8 T-NHL) showed no clonality, 8 of which yielded poor DNA quality. Skin cases although had good DNA quality, usually had low number of neoplastic cells resulting in poor PCR products.

Conclusions: DNA quality is very variable and clinical information is often not provided precluding adequate assessment of clonality findings. DNA was worse locally (decalcified marrow trephines using formic acid and Peloris system with high temperatures that can cause DNA degradation). Standardisation of fixation methods and interpretation of peaks/bands in the clinical context of the patient is essential for clonality to be informative.

P123

Kikuchi-Fujimoto Disease: A Novel Diagnosis by Transbronchial Biopsy of Mediastinal Lymphadenopathy

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Objectives: Kikuchi-Fujimoto disease (KFD) is a rare, self-limiting form of necrotising lymphadenitis that most commonly affects young Asian women, and classically presents with fever, malaise and lymphadenopathy. The cervical lymph nodes are involved in around 85% of cases, with other sites rarely involved. We report an unusual case in which an unexpected diagnosis of KFD was made via transbronchial biopsy of mediastinal lymph nodes.

Methods: A 15 year old boy of Pakistani origin presented with a 4 month history of lethargy, neck stiffness and weight loss, with fever (up to 40°C) and night sweats. Chest X-ray, Mantoux test, blood cultures and viral PCR were negative. Lumbar puncture was normal, with no acid fast-bacilli. CT showed enlargement of the deep cervical lymph nodes (PET-positive on further imaging), and mediastinal lymphadenopathy. He was transferred to our hospital for further management, with a differential diagnosis of TB, lymphoma, rare infection or autoimmune disease. He underwent transbronchial biopsy of the mediastinal lymph nodes.

Results: His biopsy showed blood clot and cores of lymph node, with focal collections of crescentic macrophages, admixed lymphocytes and prominent apoptotic debris. Immunohistochemistry demonstrated a population of CD123-positive plasmacytoid dendritic cells and granular MPO positivity in macrophage cytoplasm. The background lymphocytes were mainly CD8-positive T-cells. The features were those of KFD.

Conclusion: Involvement of deep lymph nodes is unusual in KFD, and to our knowledge, this is the first case diagnosed via transbronchial biopsy. Such biopsies often produce scanty diagnostic material, and here the detection of the characteristic immunoprofile of KFD helped confirm the diagnosis. This case highlights that KFD should be considered at sites other than the cervical lymph nodes, and demonstrates the value of immunohistochemistry in reaching a definitive diagnosis.

P124

CD30 Positive Intravascular T Cell Lymphoproliferative Disorder (ivTLPD) Presenting as a Pilonidal Abscess

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We describe a benign intravascular proliferation of atypical polytypic CD30 positive T cells, co-expressing follicular T helper cell lineage markers coincidental to local sepsis of the buttock.

A 43 year old female presented with a 5x5cm buttock abscess at the site of a longstanding palpable lump. Peripheral blood showed only a neutrophilic leucocytosis. Immunohistological examination showed large aggregates of atypical CD30 positive, ALK negative lymphoid cells expressing a pan T helper phenotype with CD7 partially downregulated. Podoplanin proved that the atypical T cells were primarily, but not exclusively, localised within lymphatic channels. A T cell receptor clone was not detected using PCR.

To find intravascular concentrations of atypical lymphoid cells is uncommon in skin biopsies and raises the possibility of leukaemia or intravascular lymphoma. Intravascular lymphoma is a rare variant of non Hodgkin Lymphoma with a minority possessing T or NK cell lineage but frequently involving skin. CD30 is a transmembrane glycoprotein and a member of the TNF superfamily involved in regulating proliferation. It is considered a reliable marker of lymphoma. Primary cutaneous CD30 positive TLPDs encompass a spectrum of biological aggressiveness and include primary cutaneous anaplastic large-cell lymphoma and lymphomatoid papulosis (LyP). CD30 can also be up regulated in activated B and T cells and it has been proposed that CD30 positive ivTLPDs are equivalent to an intravascular form of LyP. Intravascular proliferations of atypical CD30 positive T cells have been linked with chronic inflammation and abscess formation. Furthermore atypical CD30 positive TLPD expressing a CD4 positive T helper phenotype and exhibiting an indolent clinical course have been reported in the arm, trunk, neck and prepuce. Ultimately ivTLPD may require follow up based upon clinical features and natural progression due to overlapping diagnostic features.

P125

Double Immuno-Staining in Lymphoma – A Pilot Study

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The investigation of lymphoid neoplasia requires multiple sections, in our practice consisting of twelve antibodies and thirteen single stained slides. By selecting particular antibodies for double staining, spatial relationships between cell types and overall tissue organisation can be more easily visualised. This pilot study aimed to optimise the staining intensity and specificity to provide accurate diagnostic information, reduce slide number, material and consumables costs, preparation time in the laboratory and storage space to enhance cost-benefit.

Twelve antibodies were chosen and paired: kappa/lambda, CD3/CD20, MUM-1/CD10, Cyclin D1/CD30, BCL-6/CD5 and CD5/PAX-5. Firstly, these combinations were applied to normal tissue and then known tumours to optimise technique. Once the staining protocols were finalised they were run on eleven consecutive cases with conventionally stained Non-Hodgkin Lymphoma (NHL) panel requests. The slides were then reviewed by the Lymphoma team for quality and diagnostic accuracy compared to the standard single stained slides.

The results demonstrate that double staining is possible in the diagnosis of NHL. The combinations chosen have proved successful and have provided interpretable results; for example, the relationship of light chain staining in plasma cells, MUM-1 and CD10 positive cells in Diffuse Large B-cell Lymphoma proves positive. We feel time will be saved cutting sections to improve efficiency in Lymphoma investigation and reduce panel storage space by around 30%.

In conclusion the outcomes from this pilot study have been positive for medical and scientific staff, has shown that double staining in the diagnosis of NHL is possible and that optimising this protocol with a view to live diagnosis is worthwhile.

P126

Using Image Analysis Software to Map in Vitro Response to Tyrosine Kinase Inhibitors in Chronic Lymphocytic Leukaemia

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In an Age in which Novel therapies are not necessarily defined by their ability to kill malignant cells, understanding the biology of malignant cells after treatment is extremely important. This is particularly true of Ibrutinib (Pcl-32765) therapy in chronic lymphocytic leukaemia which is characterised by lymphocytosis. Imagej/Fiji image analysis software could therefore be used to analyse cell shape and grouping characteristics.

We used a novel assay in which Chronic Lymphocytic Leukaemia cells, cultured for 5 days, were seeded onto fibronectin coated glass coverslips and then had their B cell receptors ligated with goat anti human Igm. They were compared with cells simultaneously inhibited with Ibrutinib. We tested multiple staining techniques and found that using either Rose Bengal or Texas Red Phalloidin staining produced the most reproducibly analysable data when using Imagej/Fiji. We demonstrated, using the assumption that the outline of interacting cells would be larger than cells which were alone that Homotypic interactions were increased after B Cell receptor cross linking (54 groups larger than 2 cells against 35 group larger than 2 cells per 500nm X 250nm Field (P=0.019)). Nuclei were also significantly larger when cross linked. (Mean 33.23 (+-0.43) pixels without cross linking and 44.15(+-0.57) (P=≤0.0001)) suggesting increased nuclear spreading. These effects were reversed by the addition Of Ibrutinib with 18 groups larger than 2 cells per the same field(P=0.0252 compared with cross linked sells) and Nuclear size mean being 33.83 (P≤0.0001 compared with cross linked cells).

This study demonstrates a novel, easily reproducible assay to assess a variety of cellular responses to Ibrutinib therapy and suggests a method of quantifying activity both when stimulated and inhibited. This technique could easily be scaled up to further investigate cellular behaviour following Ibrutinib therapy.

P127

Dealing With Dilemmas in Thyroid Pathology

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Introduction: Despite considerable lethargy seen in FNA rates, FNA continues to still find favour as a diagnostic modality in management of thyroid nodules. The value of core biopsy in assessment of neck lymph nodes to obviously accommodate performance of immunohistochemistry has found favour amongst histopathologists, Notably those practising lymphoreticular and head and neck pathology. We report two interesting cases in which a thyroid FNA accompanied a core biopsy from an abnormal neck node for a suspected thyroid anaplastic carcinoma.

A 89 year old lady presented with a nodular thyroid mass and palpable neck nodes. Thyroid FNA was markedly cellular with sheets of oncocytic cells (Thy5). Core biopsy from neck node showed metastatic carcinoma in a background of hyaline necrosis. Immunohistochemistry showed a squamous histogenesis (CK5/6 and p63 positive). Subsequent discussion prompted a search for a head and neck squamous malignancy and a floor of mouth mass was detected to be the primary source for metastases.

The second case was a 25 year old male who presented with similar findings. Thyroid FNA showed spindly cells displaying atypia and absent colloid (Thy4). Core biopsy from neck node showed metastases in a background of amyloid. Immunohistochemistry showed results consistent with medullary carcinoma.

Conclusions: Both cases illustrate a hybrid approach of thyroid FNA combined with core biopsy of a neck nodal abnormality to establish histology. The high vascularity of thyroid precludes core biopsy sampling and this is reserved for only certain categories. In contrast, nodal core biopsy has found wide acceptance with obvious benefits. The mimicry of hyaline necrosis with amyloid, Absence of Amyloid in Thyroid FNA and the Unequivocal Diagnosis Established by immunohistochemistry in these two cases are noteworthy.

P128

Low Grade Sinonasal Adenocarcinoma, Galactin 3 Expression: A Case Report

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Objective: We report a case of low grade sinonasal non intestinal type adenocarcinomas (LGSN-NITACs), thyroid-like, in a patient having thyroid enlargement. It is a challenging case because it is a rare entity, it has marked heterogeneous morphologic appearance, the thyroid like morphology, and the concurrence of thyroid enlargement. The differential diagnosis of this case includes bland glandular proliferations of the sinonasal area as respiratory epithelial adenomatoid hamartoma (REAH), low grade sinonasal intestinal type adenocarcinoma (LGSNITAC) and metastatic adenocarcinoma of thyroid as well.

Methods: Fifty years old female Egyptian patient; presented with epistaxis and left eye proptosis.

Results: Histologically, the lesion was covered with intact respiratory epithelium. Subepitheilum showed poorly circumscribed glandular growth that was composed of numerous uniform small glands or acini arranged in a back-to-back or coalescent pattern with little intervening stroma. The glands were lined by a single layer of non-ciliated bland cubical to columnar cells with uniform, round nuclei and granular eosinophilic cytoplasm. Intraluminal mucin "colloid like" is demonstrated. Tumour cells were non immunoreactive to markers, including thyroglobulin, CK20, S100 and chromogranin were negative, while they showed positivity for CK7, CK19 and galactin3. FNAC of thyroid revealed colloid goiter.

Conclusions: First reported Galectin-3 positive LGSN-NITACs, thyroid-like case.

P129

Lymphangiomatous Polyp of the Tonsil: An Unusual Mimic of Lymphoma

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Purpose of the Study: Lymphangiomatous polyp of the tonsil is a rare benign tumour, less than 30 cases of which have been reported. Such lesions typically present with symptoms of dysphagia, sore throat or throat mass. We report a case of lymphangiomatous polyp of the tonsil as a histological mimic of lymphoma.
Method: A 16 year old male with no significant past medical history presented with a symptomatic lesion of the right tonsil and underwent excision biopsy under general anaesthetic. The specimen was submitted for histological examination.
Results: Initial histological assessment of the excision specimen showed a polypoid lesion with surface squamous epithelium overlying loose connective tissue and dilated lymphatic channels. The lymphatic channels were engorged with dense aggregates of small, monomorphic lymphoid cells. The differential diagnosis included both lymphangiomatous polyp and lymphoma. Immunohistochemical staining confirmed the presence of D2-40 positive lymphatic spaces and a mixed population of CD4 positive and CD8 positive T cells, excluding lymphoma and confirming the diagnosis of lymphangiomatous polyp.
Conclusions: Lymphangiomatous polyp of the tonsil is a rare entity, which histologically may be confused with lymphoma. Immunohistochemical staining for D2-40 is helpful in confirming the diagnosis and a panel of lymphoid cell markers may be useful to exclude lymphoma.

P130

Histopathological Examination of Asymmetrical Tonsils

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Purpose of the Study: It is now universally accepted across the UK that tonsillectomy specimens are not submitted for histological examination routinely, but only when there is some clinical indication to do so. Asymmetrical tonsillar enlargement may indicate malignancy or it can be due to many causes including repeated inflammation and chronic infection. Some researchers included additional clinical features that were common when there was malignancy in the asymmetrical tonsils. In this study we aim to evaluate the cancer detection rate in this setting including those who possess high risk clinical features such as age (> 45), pain and history of smoking.
Methodology: In total, 119 consecutive tonsillectomy cases, clinically labelled as asymmetrical tonsils were analysed. Out of these, 28 cases (Group 1) were additionally labelled with investigation for unknown primary, obvious lesion or suspicious ulcer seen. The remaining 91 cases with a sole clinical indication of asymmetry were stratified as group 2.
Results: In the first group, 7 cases had a histological confirmation of tonsillar primary (25%). While, in the second group, the histological analysis showed 78 cases with benign reactive pathology (89%) while, one case was diagnosed as malignant lymphoma (1%), 6 cases with mild dysplasia (7%), one case with moderate dysplasia (1%) and two cases were diagnosed as indefinite for dysplasia (2%).
Conclusion: Our results do correlate with the considerable agreement amongst otolaryngologists that the appearance of an asymmetrically enlarged tonsil in the presence of associated risk factors is considered an indication for tonsillectomy and histological examination given the significant rate of tonsillar malignancies in this group. Anatomical difference in the depth of tonsillar fossa and asymmetry of the anterior tonsillar pillar may give a false impression of a clinically asymmetrical or unilateral enlarged tonsil.

P131

Metastatic Adenoid Cystic Carcinoma to the Lung and Kidney: A Single Case Report

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Adenoid cystic carcinoma (ACC) is a rare, slow growing and aggressive malignancy which arises within secretory glands, primarily the salivary glands, with a predilection for perineural spread and propensity for late, local recurrence and distant metastasis. The tumour has three prognostically significant subtypes which form the basis for tumour grading; cribriform, most frequent, tubular and solid, associated with a more aggressive clinical course and metastasis. In the majority of cases (74%) the tumour has an insidious onset and patients have locally invasive disease at first presentation, which coupled with adjacent, important anatomical structures means a complete primary surgical resection is often not feasible.
Distant metastases are frequent and predominately involve the lung and bone with renal metastasis a rare occurrence. An optimal treatment regime for ACC has yet to be established and while local management with combination surgery and adjuvant radiotherapy is currently favoured there is conflicting evidence regarding the use of radiotherapy and no formal surveillance guidance for local and regional recurrence or distant metastasis exists.
We report a single case of a 52 year old male who presented in 2004 with an asymptomatic right neck mass and underwent right radical neck dissection and adjuvant radiotherapy for ACC. The patient represented in 2014 with aspiration pneumonia on a seven month history of dysphagia and dyspnea. Xray and subsequent computed tomography (CT) imaging identified large, multiple right sided lung lesions, maximum 3cm, and a 5.6cm left renal upper pole mass. CT guided biopsy of the right lung lesion and ultrasound guided biopsy of the renal mass were reported as metastatic adenoid cystic carcinoma, c-KIT negative. The patient underwent a left nephrectomy in February 2015 and is awaiting further review regarding his lung metastasis.

P132

Cytokeratin 7: A Novel Biomarker in Human Papillomavirus-Related Oropharyngeal Squamous Cell Carcinoma

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Purpose: Cytokeratin 7 (CK7) is a junctional biomarker with a SEQIKA fragment which stabilises HPV-16 E7 transcripts. We assessed the expression pattern of CK7 protein in tumour specimens from patients diagnosed with oropharyngeal squamous cell carcinoma (SCC) presenting at two major Irish head and neck centres, within the last 10 years.
Methods: Archived tumour specimens together with epidemiological data were collected from patients presenting with new primary oropharyngeal SCC at two main head and neck centres in Ireland, within the last 10 years. Briefly, DNA was extracted from tissue blocks and HPV testing carried out using SPF10 HPV PCR. HPV positive cases were evaluated using the INNO-LiPAHPV Genotyping Extratest [Fujirebio]. Immunohistochemical staining for CK7 [Clone SP52, Ventana] was performed on tissue blocks following optimisation on the Ventana BenchMark Ultra Immunostainer. Slides were analysed by light microscopy and scored using the H scoring system. CK7 expression was correlated with epidemiological data, p16ink4a positivity and HPV status. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS).
Results: There were 220 cases of oropharyngeal SCC. 41% demonstrated p16ink4a positivity and 44% demonstrated HPV positivity, with 95% of these identified as HPV-16 subtype. CK7 expression was observed in the tonsillar crypt epithelium of both normal tonsils and tumour specimens. 56% of cases were positive for CK7, with 38% of cases demonstrating H score of >60. CK7 expression in the tumour cells was significantly linked to HPV status and p16ink4a-positivity (p<0.05).
Conclusions: We present for the first time expression of CK7 in the tonsillar crypts of oropharyngeal cancers. Our results suggest that the expression of CK7 in normal tonsillar crypt epithelial cells provides a selective advantage to HPV-related carcinogenesis at this site, possible due to the unique propensity of CK7 to bind and stabilise HPV-16 E7 transcripts.

P133

Junctional Biomarkers and Immune Evasion in Human Papillomavirus-related Oropharyngeal Squamous Cell Carcinoma

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Purpose: HPV-related oropharyngeal SCC represents a distinct clinicopathological subgroup of head and neck tumours. Pathogenesis of this disease in an immune-rich site is poorly understood. There are also clear similarities to SCC of the cervix. We analysed the expression of biomarkers of immune evasion and translation of cervical junctional biomarkers.

Methods: Archived HPV-positive tumour specimens and epidemiological data were collected from patients presenting with new primary oropharyngeal SCC at two head and neck centres in Ireland over a one year period. Briefly, DNA was extracted from tissue blocks and HPV testing carried out using SPF10 HPV PCR. The INNO-LiPA HPV Genotyping Extra test [Fujirebio] was used to determine genotype. Immunohistochemical staining for CK7, GDA, MMP-7, AGR-2, PD-1 and PD-L1 was performed following optimisation. Slides were analysed by light microscopy and scored using the H scoring system (junctional biomarkers). Expression was correlated with tumour, clinical and epidemiological data. Statistical analysis was performed using SPSS.

Results: Sixteen specimens of HPV-related oropharyngeal SCC were included and five specimens of HPV-negative oropharyngeal were also stained. 15/16 demonstrated p16ink4a positivity. HPV subtypes 16 (16 cases), 33 (1 case) and 44 (1 case) were identified. Junctional biomarkers were expressed in tonsil crypt epithelium and to varying degrees in tumour specimens. Expression of PD-1 (13 cases) and its ligand (14 cases) were interpreted qualitatively, based on expression pattern, often presenting at the periphery of tumour islands.

Conclusions: We have identified markers that selectively identify tonsillar crypt cells associated with HPV oncogenic infection and which correlate with cells in the cervix where top-down differentiation in SCC occurs. Markers of the PD-1:PD-L1 immune checkpoint pathway are identified and suggest a role for this immune complex formation in immune evasion in this subgroup of SCC.

P134

This abstract has been withdrawn

P135

What is the Extent of Inter-Tumour Heterogeneity in Patients with Pancreatic Neuroendocrine Tumours?

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Introduction: Pancreatic neuroendocrine tumours (pNETs) are classified under ENETS grading system. This system uses Ki-67 as a prognostic marker. Paraffin-embedded tissue from either primary or secondary tumours may be used for the production of Ki-67 labelling index (LI). Intra-tumour heterogeneity has been observed. It is not fully understood to what extent inter-tumour heterogeneity occurs and how tumour selection, for Ki-67 staining, may affect reported patient tumour grade.

Aims: To determine the extent of inter-tumour, intra-patient heterogeneity within pNETs. To determine whether tumour selection can alter patients' tumour grade and thus predicted prognoses.

Material and Method: Patients were selected from NET database, consisting of 513 patients. 17 patients were included within this study. Patients were included if they had a confirmed pNET, multiple resected specimens, and the presence of research consent. Ki-67 staining was performed on all resected specimens meeting the inclusion criteria. 2000 cells were counted in areas of Ki-67 hotspots to produce a Ki-67 LI. Comparison was made between primary and secondary Ki-67 LIs.

Results: We have looked at 17 patients, taking into account over 75 specimens. Preliminary results suggest that inter-tumour variability exists between primary and separate secondary pNETs. Additional data will be provided.

Keywords: Ki-67, Pancreatic, Neuroendocrine, Tumour, and Inter-Tumour Heterogeneity.

P136

Frozen Section Reporting of Necrotising Granuloma of the Liver Following Percutaneous Instrumentation of The Biliary Tree: A Case Series

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Percutaneous transhepatic cholangiography (PTC) is an interventional radiological technique for both diagnostic imaging and therapeutic decompression of the proximal biliary tract in malignant distal obstruction when retrograde techniques fail. Recognised complications of PTC include sepsis, haemorrhage and pneumothorax. We describe four cases where necrotising granuloma, apparently secondary to previous PTC, has resulted in frozen section examination at the time of subsequent planned cancer resection, to exclude tumour metastasis.

Four cases of necrotising granuloma in the liver have been identified between January 2013 and February 2015. All cases were planned Whipple's procedures for pancreatic cancer where initial intraoperative evaluation revealed solitary subcapsular liver lesions. Biopsy and intraoperative frozen section examination were performed to exclude metastatic disease. All frozen sections except one were reported as showing benign necrotizing granuloma formation. The first case was initially reported as malignant and the operation was abandoned. A benign diagnosis was confirmed on paraffin sections in all four cases with the first patient undergoing successful surgical resection at a later date.

To the best of our knowledge these are the first reported cases of necrotising granuloma in the liver secondary to prior instrumentation of the liver and leading to intraoperative histological assessment. We highlight this as a potential pitfall in frozen section interpretation undertaken ahead of planned potentially curative surgery which can lead to overstaging of otherwise resectable disease or to the interpretation of a potential diagnosis of tuberculosis. These risks can be reduced with greater surgical and pathological awareness of this entity.

P137

This abstract has been withdrawn

P139

Inclusion Body Fibromatosis: A Case Report

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Inclusion body fibromatosis, also known as infantile digital fibroma, is a benign, predominantly myofibroblastic tumour primarily found on the digits of infants. Clinically, these lesions present as asymptomatic cutaneous nodules, rarely larger than 2cm in size, classically on the dorsal or dorsolateral aspect of the second, third and forth digits. They have a high recurrence rate, reported as between 61 and 75%, although this can be reduced by undertaking complete wide local excision.
We report a case of inclusion body fibromatosis in an 11 month-old boy, presenting with an enlarging, firm lesion on his left second toe. Following surgical excision, the lesion showed classical histological features of inclusion body fibromatosis - spindle cells arranged in interlacing fascicles in collagenous stroma and numerous pink intracytoplasmic inclusions. The lesion appeared incompletely excised and the patient will be kept under review on account of the high risk of recurrence.

P138

Congenital Infantile Fibrosarcoma Mimicking Sacrococcygeal Teratoma in a Ghanaian Infant: A Case Report and Review of Literature

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Congenital infantile fibrosarcoma is a rare tumour of childhood accounting for less than 1% of malignant tumours in children. Reports from sub-Saharan Africa are particularly scanty and its occurrence in the sacral region mimicking a sacrococcygeal teratoma has only been reported once outside Africa. We report the case of a 9 month old female infant thought to have a sacrococcygeal teratoma but histopathological assessment revealed a congenital infantile fibrosarcoma.

P140

Decreased Expression of the Mitochondrial BCAT Protein Correlates with Improved Patient Survival in IDH Wild-Type Gliomas

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Purpose: Gliomas represent 43% of all solid intracranial tumours and are associated with a poor prognosis. Recent studies indicated that the human cytosolic branched chain aminotransferase protein (hBCATc), which metabolises the branched chain amino acids (BCAA), was significantly upregulated in IDH1/2 wild type (WT) glioblastomas, correlated with methylation patterns in the BCAT1 promoter and is associated with a worse prognosis compared with IDH mutant gliomas. The diagnostic and prognostic significance of markers of BCAA metabolism is currently under investigation.

Methods: 64 glioma tumour samples were compared for hBCATc, hBCATm and BCKDC expression using western blotting and immunohistochemistry. DNA was extracted from fresh frozen tissue. Sanger sequencing of the p.Arg132 region of IDH1 and p.Arg172 region of IDH2 was undertaken using a 3730 DNA analyser (Applied Bio-Systems). SUMMARY: In IDH WT tumours, like hBCATc (p=0.007), the expression of the mitochondrial isoform (hBCATm) is significantly (p=0.036) expressed relative to IDH mutant gliomas. hBCATm additionally shows a more significant correlation with patient survival than hBCATc on Kaplan-Meier analysis. In IDH WT tumours, low hBCATm expression is a positive prognostic factor (p = 0.003). hBCATm expression additionally correlated with WHO grade. Although previous reports indicate that increased hBCATc occurs exclusively in IDH-WT tumours, our studies demonstrate that 30% of IDH mutant tumours express comparable levels of hBCATc. Although hBCATc alone has been suggested as a putative therapeutic target, it is important to evaluate the expression of hBCATm in glioblastomas as its expression may impact the efficacy of new treatments targeting hBCATc.

Conclusions: IDH WT high grade gliomas traditionally have a poor prognosis. However we demonstrate for the first time that relatively low hBCATm may select for a better performing clinical cohort and may be a possible candidate target for drug therapy.

P141

Identification of Molecular Subgrouping of Medulloblastoma in Egyptian Patients: An Immunohistochemical Study

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Identification of the molecular subgroups of medulloblastoma (MB) has immediate clinical relevance guiding therapeutic stratification and the use of targeted therapies. Our study aimed to apply the immunohistochemical (IHC) method using robust biomarkers; glioma-associated oncogene homolog 1 (GLi1) & β catenin trying to identify sonic hedgehog (SHH) & Wingless (WNT) active tumors. This retrospective study was conducted on 49 tissue specimens of Mb cases for evaluation of IHC expression of GLi1 and β-catenin. The studied cases showed absence of nuclear positivity of β catenin & 15/49 cases (30.6%) exhibited an activated SHH pathway pronounced by nuclear positivity for GLi1. 34/49 cases (69.4%) cases that showed absence of nuclear reactivity for GLi1 and β catenin were considered as non SHH/WNT medulloblastoma group. There were no statistical significant differences between the 2 subgroups as regards to the clinical and histopathological features. Non SHH/WNT pathway identified via the absence of nuclear localization to either GLi1 & β catenin represents a major contributor to BM in the studied Egyptian patients.

P142

Alveolar Soft Part Sarcoma of the First Rib Presenting as Pancoast Tumour

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Alveolar soft part sarcoma (ASPS) is a rare tumour that was initially described by Christopherson et al. in 1952. It accounts for 0.5 - 1% of all soft tissue sarcomas. It most commonly presents in the 15 - 35 year age group with slightly higher incidence in females than males by a ratio of 3:2. We describe a case of a 55-year old female who had a right mastectomy, adjuvant chemotherapy and radiotherapy 13 years prior to her presentation to our service with a left sided supraclavicular mass that was causing shoulder pain and left upper limb paraesthesia. Preoperative PET-CT showed a 5.8 x 4.5 x 5.5cm mass, moderately FDG avid (SUVmax 6.7), arising from the 1st rib. An MRI of the brachial plexus showed no vertebral body involvement, no vessel infiltration, but suspicion of left T1 nerve sheath involvement.
Histology of the resected rib showed morphology typical of an ASPS which had arisen in bone and extended into the overlying soft tissues. There was nuclear expression of TFE3 and the (PAS+) tumour cells also unusually expressed CD68 and HMB45 as well as vimentin and NSE.
Cases of primary intraosseous ASPS are rare. To our knowledge this is the second description in the medical literature of an ASPS arising in a rib. Chemotherapy and radiation have not proven beneficial in the treatment of ASTS, although new drug trials are ongoing. Even with en bloc surgical excision and adjuvant therapy, 5-year overall survival ranges from 20% for patients with metastatic disease to 88% for patients with local disease only at the time of presentation.

P143

Myxoinflammatory Fibroblastic Sarcoma – A Rarely Described FNA Diagnosis with Histological Confirmation

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A 38 year old female presented with an occipital mass, presumed to be a lymph node and underwent fine needle aspiration of the lesion. FNA yielded two air dried slides, upon which a diagnosis of mesenchymal neoplasm was made. The patient underwent a subsequent incisional biopsy allowing a formal histological diagnosis of myxoinflammatory fibroblastic sarcoma to be made. Myxoinflammatory fibroblastic sarcoma is a low-grade neoplasm usually occuring on the distal extremities and only rarely presents as a head and neck neoplasm. FNA is a useful tool in the diagnosis and subsequent management of head and neck neoplasia and we describe here the cytological features and subsequent histological diagnosis of myxoinflammatory fibroblastic sarcoma occuring in the occipital scalp.

P144

Giant Cell Fibroblastoma: A Case Report

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Objective: Presentation of giant cell fibroblastoma (GCF). Because of it has a dilemma of microscopic appearances; it is mandatory to differentiating it from atypical dermatofibroma (ADF), fibrous hamartoma of infancy (FHI) and vascular lesions.
Methods: Eighteen month-male Egyptian child presented with painless slowly expanding subcutaneous back swelling at the left scapular area.
Results: Histologically, the lesion is poorly circumscribed and range from cellular to myxoid in a dense to loose collagenous stroma. The tumours composed of mixture of spindle shaped or stellate cells admixed with multinucleated giant cells with occasional pleomorphism and very low mitotic index (<1 per 50 high-power fields). These cells infiltrate around adnexal structures and through subcutaneous fat. A distinctive finding is cracking artifact of the stroma simulating angiectoid spaces. These pseudovascular spaces are lacking a true endothelial lining and lined by discontinuous layer of enlarged multinucleated giant cells. Immunostains, including factor VIII, CD31, CD1a, SMA, S100 and CD68 were negative. Ki 67 labeling index is very low. All the cellular components show positive immunoreactivity for CD34.
Conclusions: First case of GCF reported in Egypt. We recommend a wide scaled study to categorize this tumour with molecularly similar lesions.

P145

An Unusual Case of a Splenic Leiomyoma

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A 60 year old man being investigated for obstructive hydronephrosis was incidentally found to have a 3.2cm splenic mass on computed tomography (CT). No lymphadenopathy was present and the mass remained stable on sequential CT and ultrasound scans.

On positron emission tomography (PET) the lesion had a low signal with moderate uptake. All haematological investigations were within normal limits including a negative Epstein Barr Virus (EBV) test. His past medical history included previous immunosuppressive therapy for inflammatory bowel disease.

A core biopsy under CT guidance was performed. The cores showed a paucicellular spindle cell lesion with bland, blunt ended nuclei, no cytological atypia and a sparse chronic inflammatory infiltrate. There was no necrosis. The spindle cells stained positive for smooth muscle actin (SMA) and H-Caldesmon indicating this to be a splenic leiomyoma.

Splenic lesions are uncommon and within their differential include, lymphoma, inflammatory pseudotumour, harmatomas and leiomyomas.[1] A splenic leiomyoma is an unusual and rare benign smooth muscle tumour with an unknown pathogenesis. They are thought to arise from the capsule and blood vessel walls of organs.[2] They have been documented in immunosuppressed states (constitutional or acquired), in those with EBV infection and in children with ataxia-telangiectasia.[2] Leiomyomas within the spleen are rarely reported in the literature, especially in those patients over the age of eighteen. In this case there was historical immunosuppression, however leiomyomas should be considered in the differential diagnosis of well- defined solitary splenic lesions.

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P146

The Impact of Roux-en-Y Gastric Bypass on Biochemical and Morphological Correlates of Glomerular Injury in an Animal Model of Type 2 Diabetes

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Purpose of the Study: The Zucker Diabetic Fatty (ZDF) rat is extensively used as a model of Diabetic Kidney Disease (DKD) associated with obesity and progressive insulin resistance ('diabesity'). This study aimed to validate qualitative ultrastructural parameters of glomerular injury in the ZDF animal model and apply these criteria to an interventional study investigating the effects of Roux-en-Y gastric bypass (RYGB) on DKD.

Methods: Superficial renal cortices were immersion-fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, processed and embedded in epoxy resin prior viewing under a Technai 12 transmission electron microscope. Glomerular basement membrane (GBM) thickness, podocyte foot process diameter (PFPD) and podocyte foot process frequency (PFPF) per unit length of GBM were determined for each group (Sham and RYGB operated ZDF fa/fa diabetic animals vs non-operated non-diabetic ZDF fa/+ lean controls). Statistical analysis was performed using a Mann Whitney U test and an unpaired t-test where appropriate.

Summary of Results: Selected TEM parameters (GBM thickness, PFPD and PFPF) demonstrated significant differences between specified Sham-operated ZDF fa/fa vs fa/+ samples, p=0.017. *Analysis of RYGB interventional study samples still in progress*. Early post-operative glucose measurements showed a significant improvement in glucose homeostasis in the RYGB group (RYGB vs SHAM, P=0.0001) occurring independently of weight loss. Urinary albumin:creatinine ratios were lower in the RYGB group vs Sham operated positive controls (P=0.0079) and were comparable with age-matched lean control fa/+ samples.

Conclusions: Preliminary findings support a beneficial role for RYGB in an animal model of 'Diabesity'. Validated ultrastructural parameters should assist in elucidating changes in podocyte activation and differentiation as mediators of the observed remission of albuminuria following RYGB surgery.

P147

P16, Ki67 and HMB45 Expression in Spitz Nevi: Comparison with Melanomas and Common Nevi

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Spitz naevus is a benign melanocytic lesion that shares many histological features with malignant melanoma. Although the morphological criteria differentiating the two entities are well established however, some cases can be challenging. Many isolated markers have been proposed to help in differentiating Spitz naevus from melanoma, albeit none has been shown to be definitive.

Aim: This is a preliminary study looking at the immunohistochemical expression of 3 markers that are known to have important role in cell cycle regulation, proliferation and melanocytic differentiation (P16, Ki67, and HMB45). The aim is provide to a combination of proteins that can help in differentiating Spitz naevus from malignant melanoma.

Methods: The study included 12 cases of Spitz naevi, 6 benign compound naevi and 6 cases of malignant melanoma. Immunohistochemical expression of p16, Ki67, and HMB45 has been accessed and compared with the morphological features of these lesions.

Results: It is noted the mean P16 expression is higher in compound and spitz naevi than melanoma (83, 91, and 36 respectively). Proliferation activity as measured by KI67 index is higher in melanoma in comparison with compound and spitz naevi (30.8, 2.8, and 1.6 respectively). HMB45 shows only junctional positivity in 9 out of 11 cases of Spitz naevi while in the other two it shows week dermal component. HMB45 is constantly positive at the deep dermal component of melanoma, albeit the staining intensity is variable.

Conclusion: The immunoprofile of Spitz naevus is different from that of a malignant melanoma. A combination of biological markers as (p16, Ki67, and HMB45), can provide a potential tool to differentiate between the two entities. Nevertheless, expanding the biomarker repertoire on a large number of cases is necessary to further establish a reliable panel to differentiate among difficult cases.

P148

Direct Immunofluorescence in a Tertiary Referral Centre: An Audit of Local Guidelines and Usage

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Direct immunofluorescence (DIF) forms an important and costly adjunct to conventional haematoxylin and eosin (H&E) histology in dermatopathology, particularly in bullous diseases and other immune-mediated diseases. We aim to assess the usage and diagnostic yield of DIF in our dermatopathology department.

134 requests for DIF on skin biopsies received over a 5 month period met the inclusion criteria. Each individual report was assessed with regard to the indication for DIF, whether DIF was deemed to be indicated or not indicated on assessment of the clinical history supplied on the request card, the results of DIF and whether DIF was contributory to the final diagnosis. We also collected data on the usage of DIF over the last 2 years to assess changes in practice.

All 134 requests for DIF were granted in line with current departmental policy. The indication categories were divided as follows: bullous 41, alopecia 4, lupus 32, vasculitis 23, dermatitis herpetiformis (DH) 15 and 'other' 19. All requests for DIF were deemed to be indicated in both the bullous and DH categories by our panel, but indicated requests varied from 8.7% to 50% in the remaining categories. In 45.5% (61 out of 134) of cases DIF was deemed to be contributory to the final diagnosis. Our analysis also showed that usage of DIF in our department is escalating, with a 23.2% increase in requests from 2013 to 2014.

Our departmental policy with regard to DIF is inclusive and operates solely on the basis of clinician request. With the increasing usage of DIF, established departmental guidelines and/or a protocol for DIF usage should be mutually agreed with dermatology colleagues in order to ensure effective use of this expensive test.

P149

Overview of Merkel Cell Carcinoma in an Irish Population

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Purpose of Study: Merkel cell carcinoma (MCC) is an uncommon but highly aggressive primary cutaneous malignancy of neuroendocrine cells with a propensity for regional and distal metastases. Due to it's rarity, information relating to it's epidemiology in an Irish population is limited, mainly owing to difficulty in gathering large patient series. Our aim was to identify all cases of MCC in our institution in a defined 10 year period and review the patient demographics compared to internationally available data.

Methods: A search was carried out on the hospital laboratory system to identify all cases of MCC from 01/01/2005 to 21/12/2014. All histology reports were reviewed and any information pertaining to patient demographics was recorded in an excel spreadsheet. A literature review was performed relating to the patient profile of MCC internationally and the results were compared.

Results: A total of 33 reports pertaining to 25 individual patients were recovered. All patients were of Caucasian Irish ethnicity. The incidence of MCC was higher in men (56% of cases, n=14) than women. The median age at diagnosis was 80 years (range 57-90). Men presented at an earlier age (median 78 years) than women (median 83 years). Regarding the anatomic site of the tumours, 64% (n=16) were on the head or face, 20% (n=5) were on the lower limb and 12% (n=3) were on the upper limb. All were on sun-exposed sites. Of note, the majority of tumours in the male population were on the head (78.6%, n=11), while the female population showed an equal distribution between the head and the lower limbs (45%, n=5 for each sub-site).

Conclusions: The subset of patients we identified show demographics consistent with published literature for US, Australian and other European cohorts. Merkel Cell Carcinoma is a disease of the elderly affecting sun-exposed sites. We note some variation in the dominant anatomic sites between genders and conclude this is due to differing environmental exposure.

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A Platform for Single Cell Manipulation and Analysis (PASCA)

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Background: A novel cell-dispensing instrument referred to as a Single Cell Manipulator (SCM) device was developed with the following features: i) rapid optical and fluorescent detection of single cells ii) generation of picoliter sized droplets encapsulating the isolated single cell and iii) printing of the single cell in an "ink-jet" like manner onto a chosen substrate. This technology was used to isolate cells of interest from i) heterogeneous mixed populations of cells, ii) co-cultures of cells and iii) clinical patient samples for subsequent downstream biological analysis.

Methods: Cells were injected into a reusable silicon dispenser chip that was coupled to a live cell camera for image capture and display of cells approaching the chip's exit nozzle. An optical detection mechanism determined the presence of single, fluorescent cells within the selected region of interest close to the chip exit nozzle. A sorting algorithm ensured that only droplets containing the single cells of interest were selected for printing to the prescribed location and user-chosen substrate.

Results: Fluorescently labelled HPV16 CaSki cervical cells were spiked into a cervical liquid based cytology sample and printed onto a glass slide using the SCM. Undifferentiated NTera2 human embryonal cancer stem cells were isolated from a mixture of differentiated and undifferentiated cells based on fluorescent tagging of the cell surface receptor, stage-specific embryonal antigen 4 (SSEA4). The thyroid stimulating hormone receptor (TSHR) was expressed in anaplastic V600E mutated thyroid cancer cell lines that were treated with the MEK inhibitor PD0325901. Treated cells were isolated using the SCM.

Conclusion: The SCM PASCA technology allows isolation of single cells from heterogeneous populations of cells and clinical samples for downstream analysis at a single cell level.

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Protein Quantification in IHC Stained Human Cell Lines Using Manual Image Analysis for Calibrated Analysis of Tissue Microarrays

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This study aims at quantifying immunohistochemistry (IHC) stained human cell lines for protein biomarkers by manual pathologist review. Staining analyses are used to calibrate tissue microarrays of tumour cores, against quantitative protein concentration allowing a systems-based data analysis.

As a proof of concept, FFPE human cell line pellets (n=13) were IHC stained for Smac protein and analysed using Aperio image analysis software. Staining quantification manually performed by pathologists provided parameters including average staining intensity, percent total cell positivity and H-score. These data were enriched by qualitative parameters pointing out possible histological artefacts. A calibration curve was plotted using H-score data and protein concentrations, previously determined by Western blotting.

The panel of cell lines provided a range of strong and weak/absent IHC staining using a highly specific Smac antibody. The calibration curve showed a strong correlation between absolute protein concentrations and manual H-scores.

Expression amounts in cell lines correlate with IHC staining intensities determined by pathologist review. The linear correlation between manual H-score and absolute protein values provides an avenue to indirectly determine protein expression. Further analysis will be performed on additional antigens and analysis outcomes will be then compared to digital results. This data will provide the basis for deterministic systems-biological data analysis approaches.

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'Lean' Laboratory Requests: A Mobile App for Molecular Test Requests

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Purpose of the Study: 'Lean' is a management framework for maximising value and minimising waste. It originated in the automotive manufacturing industry and has been utilised successfully in non-manufacturing processes. One such application in our department was the 'leaning' of the molecular test requesting process using a smart-phone app. This study will look at the potential utility of this application within the National Health Service (NHS), wherein approximately twenty different molecular test request forms are currently in use.

Methods: A mobile application to facilitate molecular test requesting was developed using Xcode and the objective C programming language. The application was built around an email based system. Patient anonymity was paramount in the design; NHS numbers are used as identifiers. The application generates a molecular test request form and can also generate a national cancer drugs fund application form for each request. Administrative staff use colour coded flags to represent the progress of each email request through the workflow process to facilitate tracking.

Summary of Results: The app reduced the administrative staff workload by reducing the number of steps and paperwork involved in the molecular test requesting process. A three-fold reduction in time taken by clinicians to request molecular tests was noted. A survey of staff involved with molecular test requesting revealed a 90% reduction in 'lost requests' after the introduction of the application.

Conclusions: We present a 'lean' method for requesting molecular tests using a smart-phone app. This application can be used to standardise molecular test request forms within the NHS along with automatic generation of a national cancer drugs fund application form for each request.

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How Do Variations In Whole Slide Scanner Hardware Components and Scanning Processes Affect Digital Image Analysis Results from Scanned Microscope Sections?

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Purpose of Study: Automated approaches for quantitative digital image analysis (DIA) of tissues are becoming increasingly popular in pathology due to advances in whole slide scanning hardware and digital imaging technology. It is essential that DIA is standardised to ensure accuracy and reproducibility of results. Very limited published data exists on the effect of scanner hardware variations on the accuracy and reproducibility of DIA results. The aim of this study was to test the following variables: variation in light source intensity during the day; presnap calibration & white balance of scanned images; variation in DIA due to debris on peripheral parts of the section or coverslip edges.

Methods: Immunohistochemistry stained sections from 3 patient samples were scanned on the same Aperio CS scanner, hourly, for 2 consecutive days to generate 15 scanned images of each sample, representing 45 images in total. All scanned images were run through Aperio software using a macro to quantify positive cell counts. For a subset of images, a region of interest was drawn around the tissue to exclude any debris/coverslip edges from peripheral parts of the slide in the subsequent DIA. Statistical analysis was performed to calculate the coefficient of variation between DIA results from scans on different days.

Results: Variation in light source intensity accounted for 1.2% to 2.1% variation in cell counts between repeat scans of the same slide. Exclusion of debris/coverslip edges accounted for 1-2% variation in cell counts between repeat scans of the same slide. Subjective analysis revealed no significant difference in appearance of different scans of the same slide.

Conclusion: Variation in light source intensity, presnap calibration and overall white balance, plus debris in peripheral areas of the section account for minimal variation in resultant DIA results. Technical advances in scanner hardware have reduced variability in scanning operations. Further investigations are ongoing.