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AIDPATH · British Lymphoma Pathology Group
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UK Cardiac Pathology Network · Renal EQA

KEY

Ⓟ = Presenter

PRESENTER'S INDEX

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P1

Incidence of Psychiatric Illnesses in Sudden Cardiac Deaths

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A retrospective study of the incidence of psychiatric illnesses among 1613 sudden cardiac death cases referred to the Cardiac Risk in Young Cardiovascular Pathology Unit at St. Georges' University of London between 2013–2016. A total of 170 (10.4%) cases with a psychiatric history were found.
Results: The frequency of cases with psychiatric history increased from 13 in 2013, to 56 cases in 2014 and 93 cases in 2015. The majority were males (61%). Highest incidence was found in 30-39 year group (31%). Psychiatric illnesses reported, depression (47%), non-specified (19%), depression with anxiety (13%), schizophrenia (11%), anxiety (7%), learning difficulties (6%), psychoses (2%) and others (8%). 95% died either at rest or sleep, 4% died during stress including 4 in police custody.Use of psychiatric medication was reported in 47%. Non-toxic levels of drugs were found in 24.7% of cases, alcohol in 30%.The commonest cause of death was sudden adult death syndrome with morphologically normal heart (68%), followed by idiopathic left ventricular hypertrophy (8%), cardiomyopathies (8%), ischaemic heart disease (6%), valvular heart disease (2%), hypertensive heart disease (2%) and other causes (6%).
Conclusion: There is a disproportionate number of sudden cardiac deaths with psychiatric illnesses and the role of stress and drugs which may effect the electrical activity of the heart must be considered. Medicolegal issues such as death in police custody are important considerations also. Autopsy with detailed cardiac examination is essential in all these cases.

P2

Strongyloides Stercoralis: A Surprise Diagnosis at Autopsy

Ⓟ SA Collis[1]; SAJ Wallace[2]; CP Johnson[1]

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Opening the bowel at autopsy may not be a pleasant task but in some cases can be extremely worthwhile. This is a case report involving an elderly man who had been suffering from acute bronchitis with underlying COPD. A subsequent autopsy identified severe emphysema with bronchopneumonia and influenza B virus. Erythematous lesions were seen in the large bowel; and following histological examination numerous worms (Strongyloides stercoralis) were discovered in the bowel wall as well as the lung and liver. S.sterocoralis is a nematode worm found in soil, endemic in tropical and subtropical regions. The worm penetrates the skin and has a complicated life cycle within the body persisting for decades. Host immunosuppression (associated with high dose steroids or HTLV-1 infection) may potentially cause autoinfection resulting in life threatening disseminated strongyloidiasis or hyperinfection syndrome. Disseminated S.stercoralis is not a common finding in routine Coronial autopsy practice in Liverpool but, has been reported by the Liverpool School of Tropical medicine previously. The deceased was originally from Sierra Leone where S.stercoralis is endemic. Identification of disseminated S.stercoralis was considered to have contributed to his death. This case highlights the importance of conducting a thorough postmortem examination even on a seemingly routine case as unexpected findings may be significant and further investigations can be valuable.

P3

How has the Introduction of the Interim Medical Examiner Group (IMEG) Affected HM Coroner Referrals, and Coronial / Hospital Consented Post Mortem Rates?

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Purpose of the Study: The Interim Medical Examiner Group (IMEG) in University Hospital Southampton NHS Foundation Trust (UHSFT) was established as a response to death certification reforms recommended by the Coroners and Justice Bill 2009. IMEG was introduced to improve accurate death certification and HM Coroner (HMC) referrals, and to review the quality of care, concerns regarding patient care, and identify clinical incidents. IMEG commenced on 1 September 2014. It reviews all adult inpatient deaths occurring in UHSFT. The IMEG review panel comprises five clinicians, four histopathologists and a member of bereavement care staff. Since its introduction, IMEG review has resulted in significant changes to cause of death formulation. IMEG has progressively reduced the hospital standardised mortality ratio, and raised points for discussion during Morbidity and Mortality meetings. The aim of this study is to evaluate the rates of patient death referrals to HMC, Coronial post mortems and hospital post mortems pre- and post- introduction of IMEG.
Methods: Data was collected retrospectively from IMEG review forms over 12 months (1 January 2015 to 31 December 2015). Referral to HMC, Coronial and hospital consented post mortem rates were compared with data from the same period pre-IMEG (1 January 2013 to 31 December 2013).
Summary of Results: Preliminary results show that out of 88% (1678 cases) of all inpatient deaths reviewed by IMEG, 40% of these were referred to HMC. 16% (270 cases) required post mortem or inquest. There was a decrease in HMC referrals (by 3%) and an increase in hospital post mortems from 23 to 29 cases after IMEG introduction.
Conclusion: Current results support IMEG introduction as being beneficial in reducing inappropriate referrals to HMC and subsequent Coronial post mortems, and increasing the number of hospital consented post mortems.

P4

Autopsy Cranial Tissue Examination is not needed unless there is a prompt to open the head

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Purpose of the Study: Some consider that only reliable examination is the 'full' autopsy, with head, thorax and abdomen as the standard; with additional tests. Yet, there is variation in the range, type and extent of UK Coronial autopsy practice. A rise in 'limited autopsy' requests prompted a review of local autopsy practice.
Methods: The autopsy reports from one pathologist, over a 15 year period, were reviewed to consider the derived value of cranial examination.
Results: There were 2195 autopsies. The head was not examined in 345 cases, often reflecting known ante-mortem investigations. Of the 1850 cases, where the head was examined internally, 590 had a specific prompt to examine the head. However, 549 of these cases had no new internal findings, although 41 of these 'prompted' cases had additional data (eg. bleeding, trauma effects, neoplasia, etc). This leaves 1260 cases without any head examination prompt. In the majority (n=1250, 99.2%) there was no additional data defined by examining the head tissues. Six cases showed pathology, without ante-mortem symptoms/effect, judged of minimal diagnostic value. The remaining cases (n=4, 0.3%) had significant intracranial bleeding (worthy of inclusion in the cause of death), as unexpected findings.
Conclusions: The study does not argue against cranial tissue examination, but it is considered that this examination component should perhaps better be seen as one of the ancillary investigations in the autopsy (akin to microbiology, toxicology, etc), and to be chosen on occasion rather than being a core item.

P5

Reporting on Ki67 Expression Utilising Core Needle Biopsy and Surgical Excision Specimens in Older Women with Invasive Breast Cancer

Ⓟ MA Albanghali[1]; MA Aleskandarany[1]; EA Rakha[1]; BM Syed[2]; AR Green[1]; C Nolan[1]; M Díez-Rodríguez[1]; IO Ellis[1]; KL Cheung[1]

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Introduction: Ki67 is a well-established prognostic marker in breast cancer (BC). Intra-tumour heterogeneous expression of Ki67 might impact its accurate reporting especially on core needle biopsy (CNB). This study aimed to evaluate the reliability of CNB to investigate Ki67 expression by comparing it with surgical excision (SE).
Material and Methods: Paired samples of CNB and full-face SE were collected from 119 older (age ≥70) women with primary invasive BC. Based on the availability of tumour tissue, multiple copies (CNB1, CNB2 & CNB3) from CNB were used to construct a tissue microarray (CNB TMA) block utilising a technique developed in our laboratory. Sections from CNB TMA and full-face SE specimens were immunohistochemically stained with Ki67 (MIB-1 clone, DAKO, Denmark).
Result: High Ki67 expression was detected in 53% (n=63) of cases using SE and 37% (n=44) using CNB. Estimated concordance between SE and cases with one CNB copy was 56%, whereas this estimate increased to 66% with expression from 2 copies and 88% with 3 copies. Estimated false negative cases based on 1 copy was 30%, 2 copies 27%, and 3 copies 0%. On the other hand, positive predictive values showed 59% (95% CI; 38.8%-77.6%) for 1 copy, 80% (95% CI; 44.4%-97.5%) for 2 copies and 86% (95% CI; 42.13%-99.64%) for 3 copies. Concordance between multiple copies of CNB, CNB1&CNB2, CNB1&CNB3 and CNB2&CNB3, were 76%, 100% and 38% respectively.
Conclusion: Sensitivity and specificity of using CNB to report on Ki67 appear to improve with multiple copies. CNB can be used to assess proliferative status of BC in older patients particularly in those where surgery is not the favourable option.

P6

Rho-GTPase Activating-protein 18 (ARHGAP18): A Biomarker Associated with Lymphovascular Invasion in Invasive Breast Cancer

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Purpose of the Study: Although lymphovascular invasion (LVI) is recognised as a crucial early step in metastatic cascade and breast cancer (BC) mortality, driver molecular pathways and potential therapeutic targets associated with LVI remain lacking. This stems from complexity of the process as well as limitations of study designs and power. We hypothesised that large-scale genomic and transcriptomic profiling of histologically validated LVI can potentially identify LVI-driver genes.
Methods: Integrative bio-informatics analysis of gene expression and copy number aberration (CNA) data was applied to identify key LVI-associated genes in strictly selected two subsets of the METABRIC study. LVI status of these cases has been validated histologically and immunohistochemically. ARHGAP18 showed significant CNA fold change between LVI+ and LVI- cases. ARHGAP18 was assessed in a large cohort of BC with long-term follow-up using IHC and TMA. The prognostic impact of ARHGAP18 gene expression was externally validated within the online BC gene expression datasets using bc-GenExMiner v3.0.
Summary of Results: Cytoplasmic expression of ARHGAP18 showed significant association with LVI, the Nottingham Prognostic Index, and luminal subtype. Nuclear over-expression was associated with lower grade, better NPI, histological types of excellent/good prognosis, HER2-, low Ki67 and luminal subtype. Cytoplasmic and nuclear expression showed significant association with longer BC survival and distant-recurrence free survival; p<0.05), independent of other factors. Using External validation cohorts (n=2,016), high ARHGAP18 mRNA expression showed significant association with longer DMFS (p<0.001, HR=0.82, 95% CI 0.75-0.90).
Conclusions: ARHGAP18 is associated with improved outcome in BC at CNA, gene expression, and protein levels. The association with LVI warrants further validation both in functional and prospective studies to have clearer insight into its exact roles in this complex process.

P7

Phosphorylated Activating Transcription Factor 2 is Related to Good Prognosis in Invasive Breast Cancer

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Purpose of the Study: The phosphorylated (p) Activating Transcription Factor 2 (p-ATF2) is transcription factor that has the ability to perform mechanistically distinct functions including cell response to stress, cell growth, and cell death. It has both oncogenic and suppressor activities in various tumour types including breast cancer (BC) although these remain controversial. Moreover, it may be also involved in DNA damage response independent of its transcriptional regulatory role. The aim of this study was to assess p-ATF2 expression in invasive BC with relevance to prognosis and its association with different molecular pathways.
Methods: p-ATF2 expression was assessed in a well-classified series of BC (n=1301) with long-term follow-up (>20 years) using tissue microarray and immunohistochemistry. p-ATF2 expression was scored using H-score and correlated with clincopathological parameters and patient outcome.
Summary of Results: High p-ATF2 (>70 H-score) was associated with lower tumour grade , lobular cancer, luminal BC, ER+ PR+, pan and p MAPKs including JNK1/2, ERK1/2, p38, and c-jun (all p< 0.001), ER related proteins [BEX1 (p=0.006), FOXA1, & GATA3 (both p<0.001)]. Low p-ATF2 was observed with HER2+ (p=0.005), p53+ (p=0.027), Ki67 (p<0.001), and with DNA repair proteins including CHK1, ATM, ATR, BRCA1, RAD51, and PARP1 (all p<0.01). High p-ATF2 was associated with prolonged BC specific and distant metastases free survival, independent on other factors (p<0.001, Hazard ratio=0.64 95%CI 0.50-0.83) in unselected BC and ER+ tumours.
Conclusions: This study demonstrates p-ATF2 as a marker of good prognostic impact, implying a potential tumour suppressor role in BC. Furthermore, the associations with proteins involved in DNA damage indicate a role in DNA damage response. These could be further investigated to supplement BC directed therapies.

P8

Breast Carcinomas with Lobular Features: A Pathological Review

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Introduction: Lobular growth pattern can be seen in breast carcinoma as pure invasive lobular carcinoma, as distinct lobular component in a mixed tumour and as lobular growth pattern in Ductal/No Special Type (NST) breast cancer. Presence of lobular pattern has been noted to affect tumour characterisitcs in terms of assessment of tumour size, choice of surgical procedure, local recurrence and behavior. We aimed to study carcinoma with lobular features and compare its pathological features with other cancers. Mehtods: Histpathology database was searched for all invasive breast cancers diagnosed between July 2008 and January 2016 which showed lobular features. Surgical excision status, tumour subtype, size, histological grade, lymphovascular invasion and lymph node status were noted.
Results: Overall 1851 cases of invasive breast cancers were diagnosed during the study period. Surgical resection was carried out in 1405 cases. Of these, 255(18%) carcinomas showed lobular features. Distribution of various subtypes was as follows; invasive lobular carcinoma 173(68%), Mixed carcinoma with a lobular component 42(16%) and NST with lobular features 40 cases (16%). When compared with carcinomas without any lobular features, carcinomas with lobular features were more likely to be larger (pT2 and above) in size (55% vs 32%), lower (grade 1&2) histological grade (88% vs 60%), ER positive (97% vs 78%), lymph node positive (36% vs 29%) and undergo mastectomy for complete excision (64% vs 54%). These tumours were less likely to show Her2 positivity (6% vs 15%) and lymphovascular invasion (19% vs 29%).

P9

Lymphovascular Invasion in Breast Cancer and its Association with Other Pathological Variables

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Introduction: Lymphovascular invasion (LVI) is seen in 15-35 % of invasive breast cancers (BC) and is thought to have prognostic significance. We aimed to study prevalence of LVI in our centre and establish its relationship with other pathological variables in BC.
Methods: Pathology computer system was searched for all cases of invasive BC reported at our institution between July 2008 and January 2016. Presence or absence of definite LVI was assessed in peritumoural tissue on hematoxylin and eosin (H&E) stained sections. LVI was defined as carcinoma cells present within a definite, endothelial lined space. Other tumour characteristics like size, histological grade, tumour subtype, ER, PR, Her2, lymph node metastases were also assessed. Correlations between LVI and other pathological parameters were investigated.
Results: Overall 1851 cases of invasive breast cancers were diagnosed during the study period. Of these, resection was carried out in 1405 cases. LVI was seen in 376 cases (27%). Frequency of LVI in various tumour subtypes was as follows; invasive micropapillary (80%), basal type (37.72%), apocrine type (33.34%), and ductal/No Special Type (28.88%), mixed cancers (28.3%), mucinous (16.67%), invasive lobular carcinoma (11.62%), metaplsatic carcinoma (10%). No LVI was seen in tubular, medullary like, cribriform, adenosquamous and adenoid cystic carcinomas. There was a positive correlation between LVI and higher pT stage, higher histological grade, and higher lymph node status ($p < 0.05$).
Conclusion: LVI in BC observed in our series, in concordance with published literature, is strongly correlated with reported histopathological variables. The strong correlations highlight the importance of LVI in BC as an important parameter to convey to the MDT.

P10

Pre-operative Diagnosis of DCIS: A Pathological Review

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Introduction: Ductal Carcinoma In-Situ (DCIS) is increasingly being diagnosed on needle core biopsy (NCB) especially in populations within a screening programme. A significant proportion (18-42%) of DCIS is upgraded on resection to invasive carcinoma. We aimed to review our experience of preoperative diagnosis of DCIS on NCB.
Methods: Pathology computer system was searched for all cases diagnosed between July 2008 and Jan 2016 as DCIS on NCB. Characteristics like NCB nuclear grade, surgical excision status, whole tumour size, resection DCIS nuclear grade, invasiveness, invasive carcinoma histological grade and lymph node status were noted.
Results: Overall 2032 breast cancers were diagnosed, of which 200 cases were reported as DCIS on NCB. One hundred and eighty one cases of DCIS underwent surgical resection; local excision (n=78, 43%) and mastectomy (n=103, 57%). Of the resected cases 51(28%) were upgraded to invasive carcinoma. Among cases which were upgraded to invasive carcinoma, there was a higher proportion of tumours with a whole size of >2cm (32% vs 17%). In cases with a final surgical diagnosis of DCIS, final DCIS nuclear grade matched with NCB grade in 75% (n=94) cases, while 18 %(n=23) were upgraded and 6 %(n=8) were downgraded. Among invasive carcinomas, higher (grade 2&3) histological grade (n=38/50) was seen more commonly in cases with a higher DCIS nuclear grade on core biopsy (92% in high nuclear grade, 67% in intermediate nuclear grade and 43% in low nuclear grade). Among 48 invasive breast cancer with axillary staging, 3 had lymph node metastases.
Conclusion: Needle core biopsy diagnosis of DCIS results in under-estimation of invasiveness in 28% of cases. Larger tumour size (>2cm) is more likely to be associated with underestimation. Histological grade of invasive carcinoma correlates with DCIS nuclear grade on NCB. DCIS nuclear grade on core biopsy correlates with final resection grade in 75% of cases.

P11

Subcellular Localisation Specific HOXB13:IL17BR Protein Index Predicts For Poorer Prognosis and Metastatic Progression in Breast Cancer

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Introduction: Gene expression analysis of breast cancer (BC) cohorts have identified novel risk predictors HOXB13 (a homeo-domain — containing protein; regulating terminal cellular differentiation) and IL17BR (interleukin-17 receptor B; Habel et al, Breast Cancer Res, 2013, 15(2)). The two-gene expression (HOXB13:IL17BR), H:I index, has been shown to predict outcome. The aim of the current study was to investigate the potential clinical utility of the H:I index at protein level in BC in context of the subcellular localisation of components.
Methods: Tissue microarrays from a well annotated series of BCs (n=960) were immuno-stained for HOXB13, IL-17BR and scored in terms of subcellular localisation. Combinations of the H:I index specific to staining compartments were investigated for differential correlations with clinical variables and prognosis.
Results: Cytoplasmic (c+) HOXB13 revealed a negative association with ductal tumours and ER/PR+ ($p=0.01$) expression but correlated positvely with grade ($p=0.0001$), p53 ($p=0.02$) and NPI ($p=0.009$). Positive nuclear (n+) IL-17BR was associated with lower grade, smaller tumour size and negative LVI status ($p=0.036$). HOXB13 (n+) and IL-17BR (c+) showed reverse trends. High H (c+): I (n+) index was associated with poor prognostic features such as larger size, pleomorphism, LVI ($p=0.04$), distant metastasis ($p=0.033$), shorter BC-specific survival (BCSS; $p=0.034$) in the whole cohort as well as ER+ grade 1 tumours. In contrast, high H (n+): I (c+) index correlated negatively with grade ($p<0.0001$) and proliferation ($p=0.007$). Multivariate analysis revealed that H (c+): I (n+) index was an independent prognostic indicator of BCSS ($p=0.044$).
Conclusions: Results from this study suggest the H:I index at the protein level may be useful to predict BC progression and patient outcome. The index needs to be refined per sub-cellular compartment to define potential clinical utility.
**Project supported by NIHR and CDF from PathSoc*

P12

Investigating the Role of CREM Protein in Lymphovascular Invasion in Breast Cancer

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Hypothesis: Lymphovascular invasion (LVI) in breast cancer (BC) is related with poor prognosis. The molecular mechanisms of LVI are still not completely understood. Through interrogation of the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) cohort, CREM (cAMP-responsive element modulator) was shown to be down-regulated in LVI positive cases ($p<0.001$; logFC -1.28). This study was to analyze the expression of CREM protein in BC and examine its relationships with LVI.
Methods: Breast cancer tissue microarrays (n=1280) were immuno-stained for CREM and expression patterns correlated with clinico-pathological and molecular variables as well as patient outcome. Results were validated in other BC cohorts [Breast Cancer Gene miner].
Results: Nuclear CREM expression was negatively associated with grade ($p<0.001$) and NPI ($p<0.001$), It was also negatively associated with N-cadherin ($p<0.001$), intra-tumoural CD68 ($p<0.001$) and FOXP3 ($p<0.001$) expression. Cytoplasmic CREM expression was positively associated with lymph node status ($p=0.02$) and LVI ($p=0.007$) but negatively associated with N-cadherin ($p<0.001$). Cytoplasmic CREM expression was also associated with poor breast cancer specific survival ($p=0.018$), while nuclear expression was related with better prognosis ($p=0.002$). Targeted prognostic analyses for CREM via the BC gene miner in ER+ node -ve patients (n = 1 419) indicates high levels of gene expression to correlate with better metastases-free survival ($p=0.02$).
Conclusions: Results indicate nuclear expression of CREM correlates with better prognosis in BC while cytoplasmic sequestration is correlated with LVI and poorer prognosis. Further functional studies deconstructing the CREM/CREB (cAMP response element-binding protein) pathway will better delineate underlying molecular events.
**Project supported by NIHR, Academy of Medical Sciences and CDF from the PathSoc*

P13

Prognostic Significance of Tumour Infiltrating Lymphocytes in Ductal Carcinoma In Situ

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Background: Tumour infiltrating lymphocytes (TILs) are an important component of the immune response to cancer as well as being prognostic and predictive biomarkers in several cancers, including breast cancer. Although several studies have investigated the role of T-lymphocytes in breast cancer, the role of B lymphocytes (TIL-Bs) remains uncertain. This study aimed to assess the role of TIL-Bs in ductal carcinoma in situ (DCIS).
Methods: Formalin-fixed paraffin-embedded (FFPE) sections of 80 DCIS cases (39 pure and 41 mixed with invasive component) were immune-stained immunohistochemically for B lineage markers CD19, CD20 and the plasma cell marker CD138. Density and localisation were assessed including relation to in-situ and invasive disease. Correlation with clinico-pathological data was performed.
Results: Higher numbers of CD19/CD20 positive cells were located in the stroma away from the DCIS compared with either intratumoural or tumour-adjacent stroma. The majority of tumours showed diffuse/scattered pattern of B cells rather than aggregates/follicles. Higher number of CD19/CD20 positive B-cells and CD138 positive plasma cells were significantly associated with DCIS necrosis (P=0.002), comedo-type DCIS (P=0.007), higher tumour grade (P=0.05), larger tumour size (P=0.02) and ER/PR negativity (P=0.03). Outcome analysis showed that strong expression and higher number of CD19/CD20 positive cells in pure DCIS cases were associated with longer disease free interval (P=0.05).
Conclusion: The better prognostic significance of B-cells suggests that the adaptive humoral immune response may have a substantial role in disease progression implying that cancer immunotherapy/vaccine strategies may be explored effectively to halt progression to invasive disease.

P14

Phosphorylation Status of MED1 Protein Results in Differential Prognosis in Breast Cancer

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Introduction: MED1 is an oestrogen receptor co-activator also known to interact with HER2. Recently it has been correlated with positive lympho-vascular invasion in breast cancer [Tumour Biol. 2015: 36(3)], being upregulated in metastases and implicated in therapeutic resistance. MED1 protein is phosphorylated at its activation site in a HER2 dependent manner. This study aimed to determine whether MED1 and phosphoMED1 at the protein level have differential correlations with clinico-pathological variables in BCs.
Methods: Breast cancer tissue microarrays (n=708) were immuno-stained for MED1 and phosphorylated-MED1 and expression patterns correlated with clinico-pathological and molecular variables. MED1 expression was probed in other BC series [BC Gene Expression Miner] and the METABRIC cohort.
Results: Immunohistochemistry on the Tenovus series showed high MED1 expression to correlate with ER negative status, triple negative/basal phenotype status (p<0.05) and increased lymphovascular invasion (p=0.02). On the other hand, high phosphorylated MED1 expression correlated with low grade (p<0.001), ER positive status (p=0.01), low NPI (p=0.008) and better breast cancer specific survival (BCSS) (p=0.002). Interrogating subsets of the METABRIC cohort, expression analysis and copy number alterations identified MED1 as being positively associated with lymphovascular invasion status (adjusted p value < 0.02). Datasets on the BC gene expression miner show that high MED1 expression is related to poorer survival, especially in ER positive BCs, even when adjusted for NPI and proliferation (p<0.01).
Conclusions: Results from this study suggest that MED1 expression correlates with ER negative status and probability of early metastatic spread. On the other hand, phosphorylated MED1 is a marker of good prognosis in BC.
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P15

Loss of Latent MMP7 Leads to Increased Lympho-Vascular Invasion and Poor Prognosis in Breast Cancer

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Inroduction: Matrix metalloproteinase-7 (MMP-7) plays a key role in the degradation of the extracellular matrix in various cancers but its role in lympho-vascular invasion (LVI) is yet to be delineated. During activation, the pro-peptide region of MMP7 is removed from its inactive form (pro-MMP7). Sequestration of the enzyme in a latent form should therefore abrogate early metastatic events like LVI. This study aimed to analyse the effects of latency of MMP7 in breast cancer (BC) including its relation to LVI status.
Methods: BC tissue microarrays (n=1035) were immuno-stained with an antibody against the pro-peptide region of MMP7, which is removed during enzyme activation and hence identifies latent MMP7 only. Expression patterns were correlated with clinico-pathological variables including LVI (assessed by microscopy+/- D2-40 staining).
Results: 470 (45%) BC cores showed high cytoplasmic expression of latent MMP7. Latent MMP-7 expression was higher in low grade BCs (p<0.001) with positive ER/PR status (p=0.001) and tubular/lobular morphology (p<0.001). In contrast, low expression of the latent form was significantly correlated with larger tumour size (p=0.03), both clinical (p=0.005) and IHC validated (p=0.009) positive LVI status, increased proliferation (Ki67 assessed) (p=0.008) and poorer NPI (p=0.008).
Conclusions: Results indicate that MMP7 expression in the latent state correlates with favourable prognostic features in BC. Relative loss of the latent form increases the probability of LVI and causes overall poorer prognosis. Further functional studies and correlations with interacting partners (TIMPs: tissue inhibitors of matrix metallopeptidases) will better delineate underlying molecular events.
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P16

Papillary Carcinoma of the Breast: Diagnostic Agreement and Management Implications

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Papillary carcinoma (PC), which is a rare type of breast cancer, comprises a heterogeneous group of tumours. The diagnostic categorisation of PC into in-situ and invasive disease remains a matter of debate. This study aims to assess the diagnostic agreement of PC among reporting breast pathologists.
Methods: 6 cases of PC included in the UK NHSBSP breast pathology interpretive external quality assurance (EQA) scheme in the last ten years were reviewed. In this scheme one representative H&E stained slide from each case is circulated to an average of 600 participants. Data on diagnostic categories were collected and slides were reviewed based on the WHO diagnostic criteria.
Results: Final diagnosis of malignancy (in-situ or invasive) was the highest in invasive PC (99% of the participants diagnosed it as malignant) followed by solid PC (94% and 95%), encapsulated PC (92% and 92%) and papillary DCIS (88%). Most cases of papillary DCIS were correctly classified as in-situ (77%) but 28% of the participants classified invasive PC cases as an in-situ disease. 24% of the participants reported encapsulated PC as invasive disease. Of the 2 solid PC cases, one showed some features consistent with the WHO description of invasive solid PC while the other one showed features of classic (non-invasive) solid PC. Both cases were reported as invasive by 75% and 77% of participants respectively. Breast specialists classification of PC as an in-situ carcinoma is more frequent than non-specialist participants and the difference was significant (p=0.013).
Conclusions: Recognition of PC as a malignant entity (in-situ or invasive) is high but concordance of its classification into in-situ and invasive disease is low. Histological features that can define invasion in PC should be better defined. These rare lesions require additional diagnostic work-up and difficult cases should trigger consensus opinion or expert referral.

P17

Clinicopathological Impact of C-C Chemokine Receptor Type 7 (CCR7) Protein Expression in Triple Negative Breast Carcinoma (TNBC)

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Purpose of the Study: Chemoattractants are potent regulatory mediators in numerous malignancies, including invasive breast cancer (BC). C-C chemokine receptor type 7 (CCR7) is a transmembrane protein normally expressed in lymphoid tissues regulating the targeted migration of lymphocytes to the lymph nodes. However, its aberrant expression in BC may promote the site-specific metastasis. This study investigated the clinicopathological interactions of CCR7 protein expression in tripe-negative breast carcinoma (TNBC).
Methods: Immunohistochemistry for CCR7 protein expression was conducted on tissue microarray (TMA) sections of a well-characterised series of TNBC (n=139). Statistical analysis was performed engaging robust archival clinicopathological data.
Summary of Results: CCR7 protein expression in TNBC was positively identified in cytoplasm (50.4%) and cell membrane (51.8%). High CCR7 expression was associated with a younger age at diagnosis (61.4%; p=0.009), premenopausal status (59.5%; p=0.022), and negative nodal status (59.8%; p=0.004). Lung distant metastasis was also correlated significantly with the cytoplasmic positive expression of CCR7 (76.9%; p=0.047). On the other hand, positive membranous expression of CCR7 was associated with higher occurrence of liver distant metastasis (75.0%; p=0.045).
Conclusions: CCR7 protein expression in TNBC may be associated with visceral metastasis in invasive BC. This preferential site-specific metastatic tropism in TNBC is possibly attributed to the subcellular localisation of CCR7 expression. Further molecular and functional studies are warranted to decipher the biological roles of CCR7 in TNBC.

P18

A Case of Adenoid Cystic Carcinoma of the Breast

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Adenoid cystic carcinoma of the breast is a rare tumour, accounting for only 0.01% of all breast cancers. It is an indolent tumour and carries a favourable prognosis. The morphology is similar to that seen in adenoid cystic carcinoma of the salivary glands, however within the breast it may be confused with cribriform DCIS or invasive cribriform carcinoma.We present the case of a 59 year old woman who had a five week history of a right breast lump. Clinical examination identified a deep seated nodularity in the upper outer quadrant of the right breast (P2). Mammography identified an ill-defined suspicious nodule and ultrasound showed a superficial area of echo poor tissue with an echogenic halo. The radiological appearances were indeterminate (M3/4, U3/4). Core biopsies were performed to exclude infiltrative lobular carcinoma. The tumour was formed of nests and islands of predominantly basaloid cells with little cytoplasm. There were sharp rounded spaces within the tumour nests. Focally, there were mucoid areas and pink hyaline material. Immunohistochemistry showed the tumour to be triple negative (i.e. no expression for oestrogen receptors (ER), progesterone receptors (PR) or HER2 receptors). The small basaloid cells showed strong positivity for p63 and the luminal cells showed positivity for CK7. The subsequent resection showed a macroscopically well-defined and pale tumour measuring 15 x 10 x 15mm confirmed as adenoid cystic carcinoma of the breast. Three sentinel lymph nodes were excised and they were histologically free of metastatic disease.We present the clinical, radiological and pathological features of this rare tumour, which is less likely to spread via lymphatics to the local lymph nodes and we raise the question of the necessity for a sentinel lymph node biopsy being part of the treatment protocol.

P19

Introduction of a Pilot System for Pre-requesting of ER and HER2 Immunohistochemistry

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Background: Oestrogen receptor (ER) and HER2 testing should be performed on all primary invasive breast carcinomas (with ER alone in ductal carcinoma in situ) to inform hormonal and adjuvant therapy. We piloted a system of automatic ER and HER2 immunohistochemistry (IHC) requesting for R4 and R5 breast lesions at the time of biopsy receipt with a view to improving turnaround times. However this may lead to unnecessary IHC testing. We analysed the preliminary results of the pilot protocol.
Methods: We performed a retrospective audit of all breast core biopsies received at Southampton General Hospital, between April 2014 and November 2015 (n = 1588).
Summary of Results: Of the 1588 breast core biopsies received, 599 were identified as R4 or R5 by the clinical information on the request forms and therefore qualified for automatic ER/HER2 IHC. 359 specimens had clinical descriptions only and were not eligible for IHC pre-requesting. Our protocol predicted B5 disease in 73% (546/746) of all biopsies and 87% (546/625) of cases received with an R category but led to IHC in 53 non-malignant and 32 B5a biopsies, with a total excess expenditure of £3293. The positive predictive value (PPV) for B5b disease was 61.0% and 92.7% for R4 and R5 lesions respectively, but only 13.4% for R3 lesions. Excluding pre-requesting of R4 lesions would have led to omission of up-front testing in 97 B5 cases, but prevented testing of 39 B1-B4 lesions, saving £1599.
Conclusions: R category is a robust indicator of which specimens will require IHC. The low PPV of a R3 lesion for invasive carcinoma lesion supports limiting pre-requesting R5 and possibly R4 lesions. Clinicians should be informed of the importance of specifying R category to allow early identification of patients eligible for ER and HER2 testing. The impact of this pilot on turnaround times should be assessed to determine whether pre-requesting provides a clinical benefit.

P20

Can Radiological R Code Breast Biopsies Reliably be used to Pre-emptively Order Hormone Receptors and Reduce Overall Reporting Turnaround Time?

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Background: Immunohistochemical (IHC) staining has been proven to be an essential adjunct in primary breast carcinomas. In 2015 the Royal College of Pathologist (RCPath) adjusted current key performance indicators within pathology, and increased the percentage of biopsies to be reported within 7 days from 80% to 90%. We looked at the correlation of radiological and pathological grading of breast core biopsies (R5 and B5) to address their current turn-around times by pre-empting the need for hormone receptors.
Method: We performed a correlation into the agreement between histological and radiological classification of breast cores (n =278). In 2014 all breast cores for a three month period (Apr-Jun) identified as U or R4/5 had pre-emptive hormone receptors ordered at specimen transfer and both laboratory and overall turnaround times were compared to the preceding three month period (Jan-Mar).
Results: Correlation data showed U/R5 biopsies had 98% agreement with equivalent B5 scores (k = 0.76). Of the 436 biopsies received during the 'pre-emptive' audit period, data shows the average TAT compared against retrospective data was not significantly improved (p = 0.306). The laboratory TAT (time taken from receipt to slide delivery to pathologist) was significantly reduced; from 3.33 days to 2.56 days (p < 0.0001). A small proportion of benign cases received unnecessary IHC.
Conclusion: We conclude that the laboratory turnaround for these specimens has now been optimised and is no longer the rate-limiting step in reporting. Whilst clear that there is very good concordance between radiological suspicion of the presence of neoplasia and histologically proven neoplasia, a degree of waste of materials may occur and it would be wise to measure this to see if the cost justifies the time saving.

P21

Metaplastic Breast Carcinoma Masquerading as Pleomorphic Liposarcoma - Case Report

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Introduction: Liposarcomatous tumours of the breast are among the rarest of mammary tumours. The differential includes malignant phyllodes tumour, primary liposarcoma and metaplastic carcinoma with liposarcomatous differentiation. We report an extremely rare case of metaplastic carcinoma with extensive pleomorphic liposarcomatous differentiation and its diagnostic work up.
Case: A 47-year-old woman presented with a lump in the left breast. Clinical examination revealed bilateral grade III breast ptosis and a 3x4cm mass in the lower outer quadrant above the infra-mammary fold. Mammography and ultrasound confirmed a well defined mass suggestive of a lipoma or mammary hamartoma. No pathological lymph nodes were identified. A core biopsy performed elsewhere was diagnosed as pleomorphic liposarcoma. A staging CT scan did not show an obvious primary tumour or evidence of metastatic disease. The patient was referred to a sarcoma and oncoplastic breast surgery centre for management and a therapeutic mammoplasty was done. Microscopically, this was a well defined, lobulated tumour comprising solid sheets of large pleomorphic and spindle cells with bizarre forms, vacuolated cytoplasm and ample mitoses. Atypical lipoblasts were easily identifiable. No biphasic pattern, glandular differentiation, or DCIS was noted. Immunohistochemistry showed few scattered CK7, AE1/3, CK8/18, Cam5.2 strongly stained cells. Cytokeratin 14, p63, S100, Melan A, -HCG, LCA, ER, PR, HER2 were all negative. The strong, though patchy, cytokeratin expression, favours the diagnosis of metaplastic carcinoma with pleomorphic liposarcomatous differentiation.
Conclusion: Pleomorphic liposarcomatous differentiation in breast cancer is very rare. It is mostly seen in malignant phyllodes tumour with heterologous element. Extensive sampling, careful search for a biphasic pattern, DCIS and/or epithelial differentiation and a panel of broad spectrum cytokeratins are essential to establish the diagnosis.

P22

Histological Grade Concordance Rate Between Diagnostic Core Biopsy and the Corresponding Excision Specimen in Breast Cancer: An experience at Addenbrookes Hospital, Cambridge

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Introduction: Breast cancer grade is a pathology key performance indicator (KPI) in the annual ABS BASO audit and all breast cancer core biopsy and excision specimen reports should provide details of tumour grade as a minimum dataset item. Histological grade is one of the key variables in decision regarding adjuvant therapy following a breast cancer diagnosis and core biopsy grade is used to determine eligibility for neoadjuvant chemotherapy (NACT). Although, there are no national standards for concordance in grade between core biopsy and excision specimens, concordance rate is reported as around 75% in published series in the literature.
Purpose of the Study: The aim was to look at the concordance rate of primary breast cancer grading on core biopsy and the corresponding excision specimen.
Methods: Retrospective primary breast cancer cases diagnosed between Jan 2013 and Dec 2013 were included. Cases were identified by doing a search for all breast core biopsies with a B5b (invasive cancer) diagnosis. Patients who received NACT and cases with too small core, or when a breakdown of the individual grade components (tubule formation, nuclear pleomorphism, mitotic count) was not available, were excluded. The data was analysed using microsoft excel to calculate concordance rate of overall grade and the three individual grade components. The percentage of cases with any change in overall grade (upgrading or downgrading) was also calculated.
Results: A total of 333 cases were included in the study. Three cases with either too small core or no breakdown of grade components provided, were excluded. There was concordance in 256 cases (77%), upgrading in 48 (15%) and downgrading in 26 cases (8%). The concordance rates for tubule formation, pleomorphism and mitosis were 75%, 75% and 61%, respectively.
Conclusions: The concordance rate (77%) of primary breast cancer grading on core biopsy and excision specimens is in line with the findings of published series in the literature.

P23

Investigating the Functional Significance of Aberrant αvβ6 and Fibronectin Expression in Myoepithelial Cells: Role in the Progression of DCIS?

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Background: Ductal carcinoma *in-situ* (DCIS) is a direct precursor of many invasive breast cancers, however, it is estimated that DCIS will progress in only 50% of cases, which has led to concerns regarding over-treatment. Thus, there is an urgent clinical need to determine which cases will progress and better stratify management. Genomic studies have indicated that DCIS tumour cells are as genetically advanced as invasive disease, with no specific stepwise changes identified. However, these studies ignored the breast microenvironment, which is complex, comprising the myoepithelial cell (MEC) population and the stroma. In the normal breast MECs exert tumour suppressive functions. In DCIS, MECs are altered, and this may compromise MEC function and contribute to progression. We previously have shown de-novo expression of αvβ6 in DCIS-associated MECs and up-regulation of Fibronectin. This study aims to evaluate the functional relevance of these changes.
Methods: Established MEC cell lines positive and negative for αvβ6, and primary MECs were used to assess the relationship between αvβ6 and FN expression, and the role of FN in αvβ6-mediated TGF-β signalling using 2D and 3D model systems, invasion assays and siRNA.
Results: β6-positive MECs exhibit higher levels of FN expression at mRNA and protein level compared to β6-negative MECs. Knockdown of either β6-integrin or FN in β6-positive MECs significantly reduced TGF-β signalling, TGF-β-mediated MMP-9 expression and invasion.
Conclusion: Expression of both αvβ6 and FN by MECs is required to generate enhanced TGF-β signalling, which mediates MMP-9 dependent tumour cell invasion. These changes could contribute to a progression signature to facilitate improved stratification of DCIS patients.
This work was funded by the Pathological Society PhD Studentship.

P24

Sentinel Lymph Node Biopsy Protocol, are Three Levels Enough? An Audit Conducted at a District General Hospital in the UK

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Current evidence suggests much variation and a lack of standardisation exists with sentinel lymph node biopsy (SLNB) protocols in the UK. At our institution, a SLNB is sectioned every 400 microns as a H&E slide until no tissue remains. Anecdotal evidence from regional breast centres nearby suggests they use less extensive protocols. Thereby, cutting multiple levels creates burden with time, cost and resources. The aim of the audit was to determine the feasibility of changing from multiple levels to a three level SLNB protocol without compromising the outcome of patient management. A retrospective audit from 2011-2015 of 48 patients who underwent SLNB and mastectomy, SLNB and wide local excision or SLNB alone. This yielded a total of 60 positive SLNB. All the reports were retrieved and H&E slides were re-examined. The total number of levels cut, size of nodes, the size and type of tumour metastases and whether ECS was present were analysed for each node. Clinical and pathological data was collected on tumour type, grade, stage and axillary clearance status. Most SLNB (93%) were detected at levels 1-3. The remaining (7%) were detected on subsequent levels. Two out of 60 were ITC, which did not alter management as it is regarded as N0 status. One out of 60 was a micrometastases which had other non-SLN positive nodes and therefore underwent axillary clearance. One out of 60 was a macrometastases which underwent axillary clearance, which was the only positive node out of 18 at axillary clearance. Fourteen out of 60 ECS were detected. Twelve out of 14 (86%) were found on levels 1-3. The remaining 2/14 were found on further on further levels. Practice was changed to a three level protocol without compromise in patient outcomes. Currently, the clinical significance of micrometastases and the management of patients with low volume (macrometastases) disease is still debated. This probably reflects the variation and lack of standardisation of SLNB protocols in the UK.

P25

A Comparative Study of Male Versus Female Breast Cancer Identifies Overexpression of eiF Signalling Pathways in Male Breast Cancer Providing Opportunities for a Therapeutic Window

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Purpose of the Study: Male breast cancer (MBC) is rare, understudied and its pathogenesis has not been fully elucidated. The presumption that the biology of MBC and female breast cancer (FBC) is identical is misleading with. Evidence indicates that this may not be the case. We aimed to identify gene expression in matched patient samples and validate this in MBC samples in TMA format (n=477) and relate to clinical outcome.
Methods: Transcriptomic analysis of MBC and FBC samples was conducted on over 21,000 genes. Using unsupervised clustering, differential gene expression in each gender was identified. Ingenuity Pathways and Gene Ontology Analysis was conducted on all differentially expressed genes. Several pathways were statistically significantly different between genders (adjusted FDR p-value, Fisher's exact test). Pathways of interest were confirmed by qRT-PCR. Protein expression of identified genes were assessed by IHC. Data dichotomisation was achieved by R.O.C curve analysis and KM survival curves generated.
Summary of Results: 735 genes were found to be differentially expressed between genders. eIf signalling was significantly over-expressed in males; p value 0.0001, FDR p value 0.0162, conformed by qRT-PCR. eIF4E and 5 were prognostically significant for overall survival in MBC (p = 0.012; HR = 1.77, 1.12-2.8 and p = 0.033; HR = 1.68, 1.04-2.74, respectively). In patients that co-expressed EIF4E and -5 survival significance was compounded (p = 0.005; HR = 2.471, 1.280-4.770). These findings remained significant upon multivariate analysis (p=0.002; HR 6.205, 1.99-19.30).
Conclusions: As mTOR inhibitors, which are already approved clinically, and target the eIF signalling pathway, eIFs -4 and -5 may offer novel predictive biomarkers in MBC. Our data are suggestive that the administration of mTOR inhibitors, such as rapamycin and its analogues, which target this pathway could be repurposed and offer the first MBC specific treatment for a subset of patients

P26

An Audit of Breast Fine Needle Aspiration Cytology (FNAC) Categorisation and Diagnostic Accuracy

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Introduction: Breast FNAC is a fast, quick and effective method, used as part of the triple assessment, for distinguishing between benign and malignant lesions. Its accuracy is heavily dependent on pathologists' technical expertise and interpretation experience. Therefore, it is vital to audit local performance against national standards periodically.
Methods: The audit was initially performed using all the breast FNAC samples analysed at University College London Hospital (UCLH) in 2012. The category of the each sample was recorded and correlated with the final histological diagnosis to calculate sensitivity, false positive (FP), false negative (FN) and positive predictive value (PPV). These were subsequently compared with the standards set out in the Guidelines for Non-Operative Diagnostic Procedures and Reporting in Breast Cancer Screening (2001). The audit was repeated in 2014.
Results: Our audit in 2012 included 436 samples. 23% of the sample was categorised as C1. Sensitivity was 88.9%; FP was 0%; FN was 0% and PPV 100%. These parameters have all met the minimum and preferred standards in the national guidelines. Our re-audit in 2014 included 553 samples. 30% of the sample was categorised as C1. Sensitivity was 85.7%; FP was 0%; FN was 3.1% and PPV 100%.
Conclusion: Our audit shows a slight deterioration in our department's performance on breast FNAC. An action plan has been produced which includes further staff training and more frequent re-audit. Nevertheless, FNAC has comparable level of diagnostic accuracy to core biopsy and we support its continued use as part of triple assessment at UCLH.

P27

A Head-to-Head Comparison of the Diagnostic Accuracy of Breast Fine Needle Aspiration Cytology (FNAC) and Core Needle Biopsy (CNB)

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Introduction: Breast FNAC is a fast, cheap and effective method, often used in triple assessment clinics, to distinguish between benign and malignant lesions. However, it has been superseded by CNB in many centres in the UK. Nevertheless, there are very few studies that directly compare the diagnostic accuracies of the two techniques on the same patients. This study aims to address this gap in the literature.
Methods: This retrospective study was performed on patients on whom both breast FNAC and CNB were performed in 2014 at University College Hospital London (UCLH). We accessed the hospital electronic pathology report system and recorded the sample category. Subsequently, there were correlated these with the final histological diagnosis to calculate sensitivity, specificity, false positive (FP), false negative (FN) and positive predictive values (PPV) of each technique.
Results: Our study included 84 female patients; median age range was 41 – 50 years. Our study shows comparable diagnostic accuracy values. Sensitivity: FNAC – 85.7%; CNB – 98.7%. Specificity: FNAC – 75%; CNB – 87.5%. FP: 0% for both. FN: FNAC – 25%; CNB – 12.5%. PPV: FNAC – 100%; CNB – 100%.
Conclusion: Breast FNAC is a fast and cheap option with comparable diagnostic accuracy to CNB. We recommend it as a valuable technique to be used in triple assessment clinics in centres with well trained staff and adequate resources to provide a quick turnaround of results.

P28

Role of Detection of Homozygous 9p21 (p16/CDKN2A) Deletion in Mesothelioma using Fluorescence in-situ Hybridization - Experience at a Specialist Diagnostic Pleural Pathology Service

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Introduction: Demonstration of homozygous deletion of the p16/CDKN2A gene on chromosome locus 9p21 using fluorescence in-situ hybridization (FISH) in mesothelial cells has been reported to improve diagnostic certainty of malignant mesothelioma (MM) when assessing atypical mesothelial proliferations in diagnostically challenging cases. We present a retrospective five-year experience of a diagnostic pleural pathology reporting practice of mesothelial proliferations, focusing on the application of immunohistochemistry (IHC) and p16/CDKN2A FISH analysis using a Vysis LSI CDKN2A/p16 dual colour probe.
Methods: All thoracic/pleural specimens (fluid and biopsy) coded as having a 'mesothelial'-related pathology over a five-year period (2010 to 2015) were identified within the hospital pathology database and a systematic review of pathology reports was undertaken.
Summary of Results / Conclusions: 516 specimens were included in the final analysis (381 pleural biopsy, 6 resection, 5 frozen sections, 2 other biopsy, 117 pleural fluid, 7 aspirate cytology). History of asbestos exposure was not provided for 326 (63%) specimens, while it was available and positive in 166 and negative in 24. IHC was performed in 455 (88%) specimens (EMA and/or Desmin used in 81 (15%)). p16/CDKN2A FISH analysis was attempted on 78 (15%) specimens with homozygous 9p21 (p16/CDKN2A) deletion being detected in 50 (39%). There were 2 borderline results, 25 negative and a single test failure. An average of 61 cells (range 60-110) were counted for each FISH study and the mean number of positive cells seen was 38 with the cut-off considered positive for reporting a homozygous deletion being 20%.

P29

Determining the Three Dimensional Architecture of Pulmonary Adenocarcinomas on a Microscopic Scale

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Three dimensional (3D) visualisation of solid malignancies may provide insight into their nature that cannot be seen with conventional techniques. Following development and optimisation of the three dimensional modelling techniques described, it may be possible to use such a method in conjunction with standard tissue and genomic analysis to refine the assessment of pulmonary adenocarcinoma. Histopathological analysis of a single pulmonary adenocarcinoma following production of FFPE tissue blocks was performed alongside immunohistochemistry and molecular analysis. H&E, Ki-67, vascular and immune markers were applied, analysed and annotated. Following annotation of tissue stained with H&E and Ki-67, the images were merged to produce stacks. These were then rendered into 3D models for each biomarker using Amira 3D software. Separate areas of tumour were tested for common mutations using IonTorrent Next Generation Sequencing technology. Rendering of the 3D H&E stained model illustrated the heterogeneous nature of the pulmonary adenocarcinoma. The tumour was split into two broad components surrounding a core of central fibrosis. The tumour exhibited a stepwise progression of invasiveness as it converged on the central scar. Clear boundaries were visible between areas of in situ growth, acinar and solid pattern. Analysis using Ki-67 and rendering of the associated model showed the highest proliferative activity was at the extreme periphery of the in situ disease, with an even distribution of cellular proliferation in the regions of solid pattern. Rendering of 3D models from histopathological images allowed the production of a simplified model of the heterogeneous growth observed in adenocarcinomas. This provided an insight into the tissue alterations involved with tumour growth. Further application of this technique improve understanding of tumour development is warranted.

P30

In situ Quantification of Genomic Instability in Lung Adenocarcinoma: Biomarker Potential and the Biological Implications

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Background: Chromosomal instability (CIN) is a hallmark of cancer and a known driver of tumour heterogeneity, evolution and progression. The CIN phenotype is recognised as a central determinant of patient outcome. This study aims to determine the prognostic value of tumour CIN status in lung adenocarcinoma.
Methods: A multiplex immunofluorescent assay (cytokeratin AE1/AE3 and DAPI) was developed and applied to primary lung adenocarcinoma tissue microarrays (n=299). Whole slide digital images were captured at 40x magnification. *In silico* image analysis was then used to identify, segment and quantify the DNA content of nuclei within tumour regions. The interquartile range (IQR) of DNA content was calculated for each case. This measure is considered reflective of the extent of CIN and aneuploidy. Univariate and Multivariate Cox proportional hazards models were used to assess the prognostic value of CIN.
Results: Univariate analysis showed that extreme CIN was significantly correlated with improved overall survival (hazard ratio (HR) = 0.53, 95% Confidence Interval (CI): 0.33-0.85, p=0.009). Multivariate analysis adjusted for covariates (age, gender, stage, mucinous differentiation, lymph node status, vascular invasion, smoking status) showed that extreme CIN was an independent predictor of overall survival (HR = 0.47, 95% CI: 0.28-0.80, p=0.004).
Conclusion: Assessment of tumour CIN status may be a useful marker of clinical prognosis and therefore support treatment and risk stratification of lung adenocarcinoma.
Acknowledgements:This project was supported by a BDIAP intercalated BSc grant.

P31

Cell-in-Cell Structures in Lung Adenocarcinoma Associate With a Poor Prognosis

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Cell-in-cell (CIC) structures, where one viable cell is present within another, have been identified in a variety of solid tumours. While their significance is uncertain, repeated occurrence in tumours may represent a process that confers a selective advantage to a malignant clone via subclonal competition. However, the biological consequences and mechanisms of formation of these structures remain unknown. 10 high-power fields (hpf) were examined from 219 consecutive cases of resected lung adenocarcinoma. A scheme to identify and quantify CIC structures was formulated and used to score the tumours. Tumours were also stained for p53 expression. In vitro coculture experiments using a variety of cancer cell lines (H1299, BxPC-3, A549 and A431) labeled green or red (via transfection with fluorescent proteins) were also conducted. Cells were seeded on coated surfaces to mimic extracellular matrix. 26% of lung adenocarcinoma cases had occurrence rates of at least 5 CIC structures per 10hpf, reaching up to 23 per 10hpf. Frequency was associated with features such as multinucleation (P<0.0001), mitoses (P<0.0001) and solid pattern (P=0.002). High frequency was also related to nodal metastasis (P=0.006) and showed a trend towards poor survival. There is an association between high CIC counts and p53 positivity in tumours (P=0.0003). CIC structures are also seen in cultured cell lines and are enhanced by extracellular fibronectin or collagen, suggesting integrins may be involved in CIC formation. Live cell imaging confirms they are formed via a cell engulfment mechanism and p53 mutant status also appears to drive CIC formation in vitro. Lung adenocarcinomas frequently contain CIC structures. They are associated with cytological grade and poor outcome. There is a clear link between mutant p53 tumour suppressor status and CIC occurrence, both *in vivo* and *in vitro*. CIC structures in cell culture systems also appear to be dependent upon signaling from the extracellular matrix.

P32

Histopathological Reporting of Mesothelioma Resection Specimens Over 10 Years From a Large Single Centre

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Mesothelioma is a uniformly lethal disease for which radical surgery has not yet been accepted as a routine treatment option, yet non-surgical treatments continue to provide only limited benefit. Our NHS Trust is a leading centre for mesothelioma surgery and holds one of the world's largest cohorts of radical resection specimens. We have recently collated clinicopathological data from over 350 surgical patients operated on over a 10 year period. All mesothelioma resections from a single surgical centre over a 10 year period were reviewed and pathological data was collated. The immunoprofiles of the tumours were reviewed and demographic data was also collected for correlation to the pathological features. 368 mesothelioma resections were received in the pathology department over a 10 year period and were included in the study. The median age was 65 and the M:F ratio 307:61. The majority of the tumours (76%) were of epithelioid subtype. On TNM staging the modal tumour stage was T3 (47%) and the majority of tumours (52%) were nodal stage N2. Calretinin was the most sensitive immunohsitochemical marker used (positive in 95%). Pathology specimens from radical mesothelioma surgery are complex and challenging cases which require specialist reporting. The data presented represents the first pathological characterisation of a unique tumour resection cohort. This data will be incorporated into a comprehensive clinicopathological database alongside patient survival information for future use alongside constructed tissue microarrays.

P33

Predominant Histological Subtype and Vascular Invasion Predict Overall Survival in Resected Lung Adenocarcinoma on Analysis of a 993 Patient Cohort

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Pathological features which are recognised to predict survival in lung adenocarcinoma include disease stage and predominant histological subtype. There is conflicting evidence regarding the presence of vascular invasion and at present this not included as a core item in the RCPath lung cancer dataset. Clinicopathological data from 993 lung adenocarcinomas reported within our centre over a 14 year period was collated. This included all data available from histopathology reports and survival data captured from a range of clinical sources. Cox survival modelling was performed. Both predominant histological subtype and vascular invasion were predictors of survival, alongside disease stage. Predominant histological subtypes solid and micropapillary pattern were associated with worst survival rates and lepidic pattern was associated with the best outcome, as previously reported. This data supports the importance of predominant histological subtype as a pathological feature and suggests that the presence or absence of vascular invasion is also a key predictor of survival for which routine reporting may be indicated. All cases included in the cohort are now undergoing comprehensive histopathological grading to determine the value of additional pathological assessments such as mitotic index and nuclear size.

P34

Solid Pattern Lung Adenocarcinomas Show Shift in Cell Metabolism from Oxidative Phosphorylation to Glycolysis using Multimarker Immunofluorescence

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Metabolism is a recently recognised hallmark of cancer due to the observed metabolic changes witnessed across cancer tissues, in particular the preference for aerobic glycolysis ('Warburg effect') over oxidative phosphorylation (OXPHOS) despite its relative inefficiency. A large set of archived lung adenocarcinomas in the form of tissue microarrays (TMAs) with matched clinicopathological data were tested for markers of both glycolysis and OXPHOS using in-situ hybridisation, tyramide signal amplification and multiplexed immunofluorescence. Hexokinase II and GAPDH antibodies were chosen to represent glycolysis and Complex 1 and ATP Synthase were chosen to highlight areas of OXPHOS. Two multi-marker assays were designed, both of which incorporated a marker of glycolysis and OXPHOS along with cytokeratin to outline the tumour compartment within each TMA core. DAPI was applied as a nuclear stain. The stained TMAs were visualised using Hamamatsu slide scanner and analysed using Visiopharm software. Visiopharm apps were designed to compare the intensity of the relevant metabolism markers within the cytokeratin defined tumour compartments. Individual lung adenocarcinomas showed a preference for either glycolysis or OXPHOS cell metabolism on multimarker staining. Correlation with clinicopathological data demonstrates a statistically significant association between increased glycolysis and the following pathological features - smaller tumour size, early stage, and less nodal or vascular invasion. In addition, preliminary data suggests that lung adenocarcinomas with an observed solid pattern have a preference for glycolysis, whereas acinar pattern lung adenocarcinomas show a negative correlation. An alteration in cellular metabolism towards glycolysis has been demonstrated in a subset of lung adenocarcinomas from a large clinical cohort using multiplexed immunofluorescence. The shift is particularly associated with histological subtypes of adenocarcinomas.

P35

This abstract has been withdrawn

P36

Engulfment and Cell Motility (ELMO2) Functions as an Oncogene that Promotes Proliferation and Migration in Colorectal Cancer

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Objective: Engulfment and cell motility (ELMO2) gene is a non-transmembrane protein tyrosine phosphatase that has come into focus as a critical regulator of multiple signalling pathways. ELMO2 maps to chromosome 20q13, a region which is amplified in 40-50% of colorectal cancer. The role of ELMO2 in colorectal cancer has not been studied previously. Thus, the effect of ELMO2 knockdown on proliferation, and motility of colorectal cancer cell lines were investigated.
Methods: To define the role of ELMO2 in human colorectal cancer, we knocked down ELMO2 using small interfering si-RNA and the knockdown was confirmed by qPCR and Western Blot. PrestoBlue assay was used to study proliferation in colorectal cell lines. Transwell migration and wound - healing assays were used to test cell motility in colorectal cancer cell lines.
Result: Knockdown ELMO2 inhibited cellular proliferation in HT29 (P value <0.0001) and SW620 (P value 0.0012) cell lines. Furthermore, knockdown of ELMO2 in HT29 and RKO inhibited cell motility (p=0.0054, 0.0061, respectively) in transwell migration assay. Wound-healing assay showed that ELMO2 knockdown inhibited cell motility in HT29 cells.
Conclusion: ELMO2 exhibited an oncogenic feature in colorectal cancer. ELMO2 enhances the cell proliferation, and motility in colorectal cancer cell line.

P37

Investigation of the Role of N-glycosylation of CD24 in Colorectal Cancer

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Background and Aims: CD24 is considered an oncogene in colorectal cancer. It is a heavily glycosylated consisting of 80 amino acids and containing two N-glycosylation sites. N-glycosylation is important in protein function and we aimed to investigate the importance of N-glycosylation of CD24.
Materials and Methods: Site directed mutagenesis was used to induce change from asparagine to glutamine in the proposed N-glycosylation sites. Mutation of the N-glycosylation sites were confirmed by sequencing and expression confirmed by Western blot. Functional assays were performed by forced expression of mutant CD24 and comparison with the wild type CD24 in cell lines RKO and HCT116. Assays included a proliferation assay by presto blue, a trans-well migration assay, and an invasion assay.
Results: Removal of each of the N-glycosylation sites of CD24 caused a reduction but not a complete inhibition of the induction of cell motility (p=0.03) compared to wild type. Removal of both sites resulted in complete inhibition of the induction cell migration, and invasion equivalent to that of the empty vector controls (p=0.003 and P= 0.004 respectively). Unexpectedly these mutations of CD24 significantly enhanced cell proliferation (p=0.0001) compared to wild type CD24.
Conclusion: CD24 mediates cell motility through N-glycosylation and mutations appear to inhibit proliferation. The mechanisms by which these alterations mediate their affect requires investigation.

P38

Cten Stimulates Cell Motility Through Src Signaling in Colorectal Cancer

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Purpose of the Study: C-terminal Tensin-like (Cten) is an oncogene in colorectal cancer (CRC) and the protein is localized at focal adhesion. It regulates cell motility although the mechanisms underlying this role of Cten in cancer are poorly described. cSrc is a proto-oncogene which regulates biological processes such as migration, and invasion. We hypothesized that Cten may stimulate cell motility through Src signaling.
Methods: Cten was forcibly expressed and knocked-down in the CRC cell lines HCT116 and SW620 respectively. In addition, Src was knocked-down following Cten forced expression. The functional effect of Cten/Src modulation was then investigated.
Results: Functional analysis following forced expression and knock-down of Cten confirmed our previous findings that Cten positively regulates cell migration and invasion. These were accompanied by changes in FAK, ILK and Snail. Modulation of Cten levels resulted in mirrored changes in Src protein levels. There was however no change in the level of Src mRNA level suggesting that Cten regulates Src at a post transcriptional level. If Src was knocked down after Cten forced expression, the functional effect of Cten was lost and Snail induction was reduced.
Conclusions: These are the first study demonstrating Cten-Src interactions in CRC and suggest that Cten may signal through Src to stimulate cell invasion and cell motility. This may contribute to tumour metastasis.

P39

Circulating Free DNA in Colorectal Cancer as a Marker of Surgical Clearance

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Introduction: Tumour cells release cellular contents into the bloodstream as a consequence of necrosis and apoptosis. These circulating free (cf) contents have potential as cancer biomarkers. We sought to investigate whether tumour derived cfDNA and cf microRNA (miRNA) could be used as a marker of tumour removal following surgery.
Methods: Circulating free DNA and miRNA were extracted from the plasma of 16 patients with colorectal cancer . Samples were collected immediately pre-operation and daily post-operation (until discharge). QMC-PCR and High Resolution Melting (HRM) were utilized to screen cfDNA for mutations in the hotspots of KRAS, PIK3CA, BRAF, TP53 and SMAD4.
Results: Mutation in the cfDNA was detected in at least one of the genes in 10 cases. Six of these showed a loss of the mutant signal in the first or second day post-operatively. The mutant signal was persistent in the remaining 4 cases for all the samples taken post-operatively. In cases where more than 1 gene was mutated, the pattern of signal loss/retention was the same for all genes.
Conclusion: Patients can be dichotomised into a group which either loses or retains mutant cfDNA following operation. Depending on the limit of detection of methodology (yet to be determined), this may provide a novel method of assessing surgical clearance and testing for recurrence.

P40

Validating a Novel Automated PCR System for use in the Molecular Diagnostics of Colorectal Adenocarcinoma

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Purpose of the Study: Testing for BRAF, KRAS and NRAS mutations is becoming routine in colorectal carcinoma (CRC) and results are used in the screening pathway for Lynch syndrome, for prognostication and to guide targeted therapy. Conventional polymerase chain reaction-based (PCR) and next-generation sequencing-based (NGS) assays are expensive, require specialist staff and facilities and incur long turnaround times. A new PCR platform (Idylla, Biocartis) offers fully automated and on-demand processing of formalin-fixed, paraffin-embedded (FFPE) samples in a fast and cost effective manner. We undertook a validation study for the Idylla system in detecting BRAF, KRAS and NRAS mutations in CRC.
Methods: 120 tests on 80 FFPE CRC resection cases were performed on the Idylla system using the Idylla BRAF Mutation test, KRAS Mutation Test and the NRAS-BRAF-EGFR S492R Mutation Assay and the results were validated against conventional standard care PCR (Cobas, Roche), an NGS target gene panel (Ion Torrent, Thermo Fisher). The Idylla BRAF results were also compared with VE1 (Roche) immunohistochemistry (IHC).
Summary of Results: The concordance between Idylla, Cobas and Ion Torrent was 100% in all cases. When comparing Idylla with IHC the concordance was 90% but the same cases equally showed only 90% concordance between IHC and Cobas suggesting poor performance of IHC. There were no failed tests or ambiguous molecular results.
Conclusions: This study validates the Idylla technology in the detection of BRAF, KRAS and NRAS mutations in FFPE CRC tissue. The system is fast and easy to use, taking up little space in the laboratory and requiring no specialist skills to run.

P41

Sensitivity and Specificity of Automated PCR (Idylla) in the Detection of BRAF Mutations in Colorectal Carcinoma

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Purpose of the Study: Testing for BRAF mutations in colorectal carcinoma (CRC) is used in the screening pathway for Lynch syndrome and is of prognostic value to guide management. Conventional polymerase chain reaction-based (PCR) and sequencing-based assays are expensive, require specialist staff and facilities and incur long turnaround times. A new PCR platform (Idylla, Biocartis) offers fully automated and on-demand processing of formalin-fixed, paraffin-embedded (FFPE) samples in a fast and cost effective manner. This is a validation study of the Idylla Platform to determine its sensitivity and specificity for use in detecting BRAF mutations in CRC.
Methods: 100 consecutive, clinical FFPE CRC resection cases were tested for BRAF mutations using the Idylla automated platform with the Idylla BRAF Mutation Test and compared with a conventional standard care PCR (Cobas, Roche). Discordant tests were re-assayed using a highly sensitive droplet digital (ddPCR) PCR assay (Bio-Rad).
Summary of Results: Only one discordant Idylla positive / Cobas negative result was identified, which on ddPCR demonstrated a mutation not identified by traditional PCR in. Only two cases failed to give a result on the Idylla system. The sensitivity and specificity of BRAF mutation detection by Idylla was therefore 100%.
Conclusions: This study has demonstrated high accuracy of the Idylla system for BRAF testing in CRC and suggested possibly greater sensitivity than conventional PCR, in addition to cost-effectiveness and shorter turnaround time, when compared with standard PCR.

P42

Sensitivity and Specificity of Automated PCR (Idylla) in the Detection of KRAS Mutations in Colorectal Carcinoma

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Purpose of the Study: Testing for KRAS mutations in colorectal carcinoma (CRC) is of prognostic value and guides the use of targeted anti-EGFT therapy in Stage IV disease. Conventional polymerase chain reaction-based (PCR) and sequencing-based assays are expensive, require specialist staff and facilities and incur long turnaround times. A new PCR platform (Idylla, Biocartis) offers fully automated and on-demand processing of formalin-fixed, paraffin-embedded (FFPE) samples in a fast and cost effective manner. This is a diagnostic test accuracy study of the Idylla Platform for use in detecting KRAS mutations in CRC.
Methods: 30 consecutive, clinical FFPE CRC resection cases were tested for KRAS mutations using the Idylla automated platform with the Idylla KRAS Mutation Test and compared with a conventional standard care PCR (Cobas, Roche). Discordant tests were re-assayed using a highly sensitive droplet digital PCR (ddPCR) assay (Bio-Rad).
Summary of Results: Two discordant Idylla positive / Cobas negative results were identified. ddPCR demonstrated a mutation not identified by traditional PCR in one case, while one case was confirmed to be wild type for KRAS. The sensitivity and specificity of the Idylla BRAF Mutation were therefore 100% and 93%, respectively.
Conclusions: This study has demonstrated 100% sensitivity and high specificity of the Idylla system for KRAS testing in CRC, in addition to cost-effectiveness and shorter turnaround time, when compared with standard PCR.

P43

Use of HPV CISH as a Marker to Determine Site of Origin in Metastatic Carcinoma of Unknown Primary

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Use of immunostaining is well established for determination of the primary site of origin of metastatic carcinoma. To date, in situ hybridisation methodologies have not been used for this purpose. We recently instigated HPV chromogenic in situ hybridisation (CISH) with the INFORM HPV III Family 16 Probe system that can detect high risk HPV genotypes genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66. Its utility is illustrated by the case of a 47 year old lady from whom we received a lymph node with the rather brief clinical details "groin lymphadenopathy, right groin lymph node". Initial histological sections showed a lymph node containing a moderately differentiated metastatic squamous cell carcinoma. Immunostaining demonstrated that the neoplastic cells were positive for cytokeratin AE1/3, 34betaE12 and oestrogen receptor (which was positive on around 40% of the cells). Occasional cells were positive for cytokeratin 7 and progesterone receptor. The neoplastic cells were negative for CK20, TTF1, S100, melan A and HMB45. Periodic acid Schiff diastase (PASD) staining for mucin was negative. We were then made aware that the patient had a previous history of squamous cell carcinoma of the uterine cervix. We undertook CISH for HPV, which was strongly positive in the majority of cells, confirming that this is cervical squamous cell carcinoma. This case demonstrates the potential utility of CISH-based tests like HPV in determining the likely primary site of origin of metastatic carcinoma, in a manner analogous to immunostaining.

P44

The Perils of Banking Material for Molecular Analysis from Small Biopsies: A Hypothetical Analysis of Tissue Banking from Diagnostic Core Biopsies of Lymphoma

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Next generation sequencing (NGS) is being increasingly used for genomic analysis. The next goal with this technology is its application to whole genome sequencing of cancer samples in clinical practice. For diagnostic core biopsies, this could potentially involve the removal of one or two cores from core biopsies prior to formalin fixation to avoid taking additional biopsy material specifically for sequencing. Giving up these cores could affect the diagnostic utility of the specimens for histopathological examination. To assess the effect of removing cores, we undertook a hypothetical study using archival core biopsies of lymphomas (previously reported as diagnostic) in which 3 specialist haematopathologists determined whether the removal of 25% or 50% of the material from these biopsies would render them non-diagnostic. The mean percentage of slides rendered non-diagnostic with 25% of the material removed was 9.48%, while if 50% were removed, it was 19.57%. However, when we considered the slide as non-diagnostic if only one specialist deemed it non-diagnostic, the mean percentages reached 22.02% and 30.28% for removal of 25% and 50%, respectively. It is difficult to justify giving up cores in a situation in which up to 30% of patients might require repeat biopsy to confirm their diagnosis. Some diagnoses (e.g., nodular lymphocyte predominant Hodgkin's lymphoma, T-cell rich large B-cell lymphoma and T-cell lymphoma) appear more likely to be affected than others. However, as the exact diagnosis is not known prior to biopsy, it is difficult to predict which cases these will be. We discuss potential solutions to this difficulty. Our data also have applicability to solid tumours, where similar studies are required.

P45

A Next-Generation Thoracic Cancer Discovery Platform with Creation of a Large Single-Centre Retrospective Tumour Archive

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Introduction: Leicester University Hospitals NHS Trust is a busy thoracic surgical centre, with around 200 lung resections for cancer being performed annually. Tissue microarrays (TMAs) facilitate the simultaneous application of in situ assays (immunohistochemistry, immuno-fluorescent and fluorescence in situ hybridization) to numerous samples. This project aims to establish a pipeline for the acquisition and assembly of clinical data, by building a digital archive of whole tissue section images, constructing a comprehensive TMA collection with accompanying clinicopathological and follow-up data on 2000 NSCLC patients from a single surgical centre. The aim is to address biological questions about cell biological and genomic heterogeneity. Generic ethical approval for retrospective tissue access was obtained. Cases (adenocarcinoma, squamous cell carcinoma and mesothelioma) for inclusion were identified by application of specific criteria. Clinicopathological data are compiled from the pathology database, the National Cancer Registry, and local databases. Cases are anonymized, and a custom database using the RedCap application has been established behind the NHS firewall. For each case, archival slides are scanned, optimal tumour blocks identified, and TMAs containing 3x1mm cores from different regions are constructed.

Results: We have accumulated cases into our physical TMA collection, and 540 cases so far have been incorporated. These arrays contain a generous amount of tissue to facilitate manual and automated analyses, and also enable some description of pathological heterogeneity.

Conclusion: This retrospective tumour collection represents an invaluable platform for biomarker discovery, biological hypothesis testing, and hypothesis generation. It is invaluable for the application of quantitative multiplex assays. The use of digital pathological techniques has facilitated archive generation and added an extra dimension of image data.

P46

Primary versus Metastasis using Iontorrent Next Generation Sequencing (PRIMINGS)

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Cancer patients who have undergone tumour resection with curative intent may present subsequently with a tumour nodule at a distant anatomical site. For planning treatment and prognosis, it is vital to know whether this represents a metastasis from the original tumour, or a new primary cancer. Typically a biopsy of the second tumour will provide sufficient information on conventional histology and immunohistochemical profiling to determine whether this represents a new primary or metastasis. There are tumour types and tissue types in which the sensitivity of immunohistochemistry is limited however and not infrequently a diagnosis is given on the 'balance of probability'. Targeted next generation sequencing (NGS) has been shown to be useful in case reports of difficult cases but has not been tested on a series of clinical cases with a definitive diagnosis of secondary primary or metastatic tumour. A series of patients were selected from the recent diagnostic archive representing those with unequivocal dual primary tumours and confirmed primary and distant metastatic tumours. DNA was extracted from both tumours and uninvolved lymph node tissue and tested using the Ion AmpliSeq™ Cancer Hotspot Panel v2. Sequencing data was used to predict whether the second tumour represented a second primary or metastasis, blinded to histopathological and clinical information. Pairs of primary and metastatic tumours showed common mutations, with sufficient overlap to predict confidently their shared clonal origin. The pairs of separate primary tumours showed differing mutational profiles with no overlap to suggest metastasis. Testing germline DNA from uninvolved lymph node tissue allowed the exclusion of single nucleotide variants from the analysis and therefore improved the specificity of the test. In patients presenting with a second distant tumour following cancer resection, targeted NGS has the potential to be a useful clinical test to identify this as a metastasis or primary.

P47

The Use of MGMT Promoter Methylation Testing in the Management of High-Grade Glioma in South-East Scotland

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Background: : Methylguanine methyltransferase (MGMT) is expressed in tissue to confer a degree of resistance to DNA damage, specifically that induced by alkylating agents. The advent of profiling the methylation status of the MGMT gene promoter has allowed for a clinical predictor of response to alkylating agents such as temozolomide. This test is currently performed on all patients diagnosed with high- grade glioma.

Aims and Methods: This retrospective audit aimed to identify the importance of the test in the management of 196 patients with high-grade glioma, in the context of local guidelines and SMC licensing information. The behaviour of borderline methylated patients was also compared to the cohort relative in order to review the current reference value.

Results: : This study showed that, while a methylated status was significant for increasing the likelihood of treatment with temozolomide, there were sub-populations in which the test appears less helpful. In particular, these are younger patients and those with high performance status. Additionally, guidelines do not recommend temozolomide as treatment of WHO grade III gliomas. Furthermore, analysis of borderline patients was inconclusive.

Conclusions: It may therefore be advisable to restrict the testing of from the aforementioned groups apart from in the presence of explicit clinical need.

P48

Disease Progression in EGFR Mutated Lung Adenocarcinoma on Anti-EGFR Treatment

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Purpose of the Study: Non small cell lung cancers can harbour an EGFR (epidermal growth factor receptor) mutation which is detected by PCR (Polymerase chain reaction) testing of tumour tissue /cells. Patients with non-small cell lung cancers with sensitizing EGFR mutations are treated with targeted anti EGFR agents. After an initial response some patients may show disease progression due to an acquired genetic mutation and/or a morphological transformation into small cell carcinoma or very rarely to a squamous cell carcinoma.

Methods: We present a small cohort of four patients who were originally diagnosed to have adenocarcinoma on biopsy samples with a variety of EGFR mutations sensitive to TKIs (Tyrosine kinase inhibitors) in exons 19 to 21. All four patients were started on treatment with an anti-EGFR agent (Erlotinib). After a variable amount of time, they showed reduced responsiveness to treatment and disease progression.

Summary of Results: Three of the patients had stage 4 disease at first presentation and the third developed bone metastases. These patients were started on TKIs (Erlotinib). Following initial good response disease progression was noted on follow-up. Rebiopsy showed either morphological transformation or an additional genetic mutation which made the tumour resistant to the Erlotininb. The morphological transformation for one of the patients was to a small cell carcinoma, the morphology of the tumour in the second patient changed to squamous cell carcinoma and the other two patients developed a genetic mutation (T790M) imparting resistance to Erlotinib. Disease progression whilst on anti EGFR treatment in most instances is secondary to T790m mutation conforming resistance to the drug or morphological transformation to small cell carcinoma. We report an additional rare finding i.e transformation to squamous carcinoma as a cause of resistance. We are only aware of one other case of squamous transformation in the English literature so far.

P49

This abstract has been withdrawn

P50

CD24 Modulates Focal Adhesion Molecules and Regulates Cell Functions in Non-Small Cell Lung Cancer

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Background and Aim: The cluster of differentiation-24 (CD24) is a heavily glycosylated protein which is linked to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor. CD24 has been identified as a stem cell marker in different types of human cancer, and its expression has been found associated with poor prognosis. Little is known about the biological role of CD24 in non-small cell lung cancer (NSCLC). We aimed to investigate CD24 function in.
Methods: CD24 levels were modulated through either forced expression or RNA interference in NSCLC-derived cell lines. Various functional assays were also carried out to evaluate the effect of manipulating of CD24 expression on cell behaviour. Western blotting was used to evaluate changes in the expression level of potential downstream targets.
Results: Noticeable increases were seen in cell migration, invasion and proliferation following CD24 forced expression (modifications ($p<0.05$ for all) and these were all down-regulated following CD24 knockdown ($p<0.05$ for all). These data are similar to our data in colorectal cancer although we have not previously found an effect on cell proliferation. Similarly consistent with previous data, CD24 was found to be a positive regulator of expression of several downstream targets including Cten, ILK and FAK expression.
Conclusion: CD24 modulates cell function in NSCLC most probably through up-regulating Cten, FAK and ILK. There appears to be a generally consistent functional role and signalling pathway of CD24 in different tumour types.

P51

CD24 is Significantly Involved in Pancreatic Cancer Progression by Targeting Focal Adhesion Molecules and Regulating Cell Functions

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Background and Aim: The cluster of differentiation-24 (CD24) is a heavily glycosylated protein which is linked to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor. CD24 has been identified as a stem cell marker in different types of human cancer although little is known about the biological role of CD24 in pancreatic cancer.
Methods: CD24 was forcibly expressed in the pancreatic cancer cell line Panc-1 cell line whilst it was knocked down in cell lines PSN-1 and COLO357 cell lines. This was followed by assays to assess motility and invasion. Changes in expression level of potential downstream targets were evaluated and the prognostic significance of immunohistochemical CD24 expression was evaluated in a series of 81 patients with pancreatic ductal adenocarcinoma (PDA).
Results: Using our dual approach, CD24 was found significantly induce cell motility (both cell migration and cell invasion, $p<0.05$ for both). CD24 was found to up-regulate several downstream targets including Cten, ILK and FAK and markers of epithelial-mesenchymal transition (EMT). High expression of CD24 in PDA was associated with poor differentiation ($p=0.03$) and poor survival ($p<0.001$).
Conclusion: CD24 modulates cell function in PDA and it is able to induce EMT. This may be mediated through FAK and ILK signalling. CD24 is associated with a poor prognosis in PDA and may represent a new therapeutic target / prognostic marker.

P52

CTEN is Downstream Target of CD24

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CD24 is a small heavily glycosylated protein present in special docking sites in the plasma membrane(lipid rafts) attached to GPI (Glyco phoshatidyl inositol linked) proteins.CD24 has been shown to regulate many cellular properties like increasing cell motility, increasing tumour cell invasion, increased colony formation etc. However, as yet, not much is clear about its signalling partners and pathways. We sought out to explore the signalling and functional relationship between CD24 and Cten (a member of tensin proteins i-e TSN4)as both were observed to have same downstream signalling partners and both are linked to focal adhesions and cellular motility. We used co-immunoiprecipitation, co-transfections, western blotting and trans-well migration assays techniques in this study.In summary we observed that CD24 up regulation resulted in up regulation of Cten, also knock down of CD24 resulted in down regulation of Cten levels. Functional studies showed that CD24 appears to increase cellular motility via Cten as knockdown of Cten resulted in loss of increased cell motility by the CD24 in colorectal cancer cell lines. The Co-IP results, however, showed that these two molecules did not physically interact with each other. However, this does not rule out the possibility of signalling relationshipbetween the two molecules, that might still be happening through an intermediate partner .In conclusion we propose that CD24 appears to be regulating Cten levels and although it might not be the sole regulator, this regulation appears to have significant functional relevance as well.

P53

CD10 Increases Cell Invasion in Colorectal Cancer

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Cluster of differentiation 10 (CD10) is a zinc-dependant membrane metalloproteinase which cleaves a number of substrates to regulate cell signalling. CD10 has been suggested to play a role in epithelial to mesenchymal transition (EMT) pathways and it is possible that it may cleave surface molecules including E-cadherin. Since CD10 expression is associated with colorectal cancer metastasis, we investigated the role of CD10 in colorectal cancer; its involvement in cell motility and EMT signalling pathways. Following screening of CD10 expression across a number of colorectal cell lines, siRNA was used to knock down CD10 in high expressing cell line SW620. The ability of CD10 to regulate the functional activity of SW620 cells was investigated using the PrestoBlue proliferation assay, Transwell migration and invasion assays. Western blot analysis of an EMT marker panel was performed to determine underlying signalling mechanisms. CD10 expression was analysed across 84 cases comprising normal and colorectal cancer tissue. Knockdown of CD10 had no effect on cell proliferation or migration however knockdown was associated with a decrease in cell invasion ($p=0.0101$). Knockdown of CD10 was not associated with changes in the expression of E-cadherin, N-cadherin, Vimentin or Snail and therefore it may not regulate EMT signalling pathways. Analysis of CD10 expression in colorectal tissue revealed positive staining in 31% cases however expression was not associated with tumour stage, vascular invasion, Dukes' stage or lymph node involvement. We have demonstrated that CD10 increases invasion in colorectal cancer however the knowledge of underlying signalling mechanisms is sparse. Further investigation may uncover novel invasion signalling pathways in colorectal cancer. This work was funded by a Pathological Society grant.

P54

Should ER Status be Repeated on Surgical Excision in the Case of ER Negative Breast Core Biopsy?

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Purpose of the Study: Oestrogen receptor (ER) status is a crucial predictor of response to hormonal neoadjuvant and adjuvant therapy in patients with breast carcinoma. The concordance between core biopsy ER status and subsequent surgical excision is thought to be high, with rates reported in the literature of 86-100%. We evaluated both the rate of re-assessment of ER status on excision in cases where initial immunohistochemistry had demonstrated ER negativity (defined as a Quick score of 2/8 or less), and concordance between ER negative core biopsies and ER status at surgical excision.
Methods: All ER negative invasive breast carcinoma (B5b) core biopsies diagnosed at the University Hospital Southampton NHS Foundation Trust between April 2014 and November 2015 were identified and subsequent surgical excision ER status recorded.
Summary of Results: 77 ER negative core biopsies with excision data were identified. Repeat ER testing was performed on 58% (45/77) of cases. Concordance for ER negative status between core biopsy and surgical excision was 98% (CI 94-100%). In one case the tumour was subsequently found to be ER positive on surgical excision (Quick score 5, confirmed on review).
Conclusions: Our rate of repeat assessment of ER status on surgical excision specimens was low. Although the concordance between ER negativity on breast core biopsy and excision was high and comparable to previously reported rates in the literature, to reduce the potential for inappropriate exclusion of hormonal treatment our findings suggest that ER status should be repeated on excision specimens if prior core biopsy ER status is negative.

P55

General Autopsy Histology: An Online Resource

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Introduction: In modern day practice the taking of tissue samples for microscopic examination during adult post mortem examination is becoming infrequent and as a result trainee and consultant histopathologists have less exposure to post mortem histology. Tissue sampled at post mortem can display changes not usually seen in surgical specimens and is often taken from organs in quantities not present in daily surgical histopathology practice (e.g. full thickness sections of myocardium). Therefore it is important that trainees and consultants have access to collections of slides taken at post mortem.
Aim: In 2015 the authors were awarded a Pathological Society Educational Grant. It enabled collaboration with PathXL to begin to digitalise a unique slide collection of post mortem histology, creating an instructive atlas of images as an online educational and reference tool. A case-based format was selected with emphasis on clinicopathological correlation. The resource is incorporated into the secure Pathological Society Education Portal. The authors have access to a large and continuously expanding archive of post-mortem histology slides, all anonymised and fully consented for education under the Human Tissue Act.
Results: We wish to demonstrate some of the cases we have digitalised to promote the resource and receive feedback and developmental ideas from the trainee and consultant delegates at the conference.
The future: Currently there is no comparable online (or text based) resource dealing with general post-mortem histology. We believe the successful development of this resource will provide content of great utility to Pathological Society members.

P56

Audit of Clinicopathological Information of Referred Placentas as per Royal College of Pathologists Guidance

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Introduction: The Royal College of Pathologists dataset has outlined the minimum clinical information for referral of placentas and suggested a triage system for examination based on the clinical situation. This study aimed to examine the completeness of clinical information and how a triage system would affect the final diagnosis.
Methods: The histology reports of all placentas received in the department from February 2015 to January 2016 were analysed. RCPath dataset was used as a standard for auditing the clinical and pathological information.
Results: 73 referrals were received in a year with 44% containing no gestational age, 98% no birth weight, 3% no clinical information/indication, 98% no information of previous pregnancies and 93% no information on maternal disease. 33 (45%) of the placentas would not have had histology based on the triage system. 2 of the 33 (6%), which would have had macroscopic examination only, and 1 of the 33 (3%), which would have been stored with no examination, were found to have an abnormality requiring histological confirmation. In comparison, 15 (39%) of the 38 placentas where histology was indicated were abnormal. 24 (33%) of the 73 referrals were twin placentas, of which the type was established correctly macroscopically in 19 (79%) cases, whilst in 2 (8%) cases histology changed the initial impression of twin type. However, there was discordance with clinical information in only 1 case.
Conclusion: Placental examination is an important part of perinatal medicine but workload can potentially be safely streamlined by a triage system of examination based on clinical situation. However, better clinical information is required for the triage system to function and to improve the examination and reporting of placentas.

P57

Assessment of the Accuracy of Depth Measurement in Large Loop Excision of the Transformation Zone (LLETZ) Specimens

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Background: The depth of LLETZ specimens is important. Overtreatment is associated with increased risk of preterm delivery whilst undertreatment is associated with increased risk of residual disease.
Purpose of the Study: To determine the accuracy of macroscopic tissue depth measurement of LLETZ specimens in our Department.
Methods: The archive at our institution was searched and all LLETZ specimens received in February and March 2015 were identified. Every third case was selected giving a total of 34 cases. All slides were reviewed and the depth of the largest section was measured on the glass slide using a ruler and compared to the original gross description. The results were presented at an educational meeting attended by pathology trainees and consultants. The importance of accurate depth measurement was emphasised and possible reasons for discrepancies discussed. The same audit was repeated post education.
Results: Of the initial 34 cases, gross measurement and glass slide measurement of depth differed by ≥ 5mm in 3 cases (9%), by 2-4mm in 9 cases (26%), and by ≤ 1mm in 22 cases (65%). The gross depth was greater in 14 cases (41%), less in 11 cases (32%), and equal in 9 cases (26%). After targeted education, in a further 34 cases, gross and glass slide measurements differed by ≥ 5mm in 1 case (3%), by 2-4mm in 16 cases (47%), and by ≤ 1mm in 17 cases (50%). The gross depth was greater in 23 cases (68%), less in 6 cases (18%), and equal in 5 cases (15%).
Conclusions: Despite targeted education, the gross measurement of depth was inaccurate. We recommend that the depth measurement is checked against the glass slide at the time of sign-out and amended where appropriate.

P58

Cardiff Pathology Careers Day — Can You Cut It?

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Introduction: On Friday 16th October, 2015, Cardiff hosted its first ever pathology careers conference. The conference was aimed at sixth form students in South Wales, and the conference objectives were to raise awareness and understanding about the roles of pathologists, and to spark an interest in the specialty so that it may hopefully be considered as a career option by the students in the future.
Results: The conference was a great success. 86 students from 15 schools attended. 85% of the students said that they had enjoyed the day, and 86% would recommend it to a friend. 93% felt that the conference had led to a greater knowledge and understanding about a career in pathology. Analysis of the pre- and post-course questionnaires demonstrated that the students increased their awareness about what pathology involves, and what subspecialties exist within it.
Conclusion: The pathology careers conference was very successful in increasing awareness and understanding about a career in pathology, and in inspiring sixth form students to consider this avenue as a future career option. Future conferences would include more interactive activities.
This conference was made possible thanks to an engagement grant from the Pathological Society (£2257.70).

P59

Recent Findings Regarding the Utility of Endoscopic Ultrasound-Guided Fine Needle Aspiration in the Grading of Pancreatic Neuroendocrine Neoplasms

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Introduction: Endoscopic ultrasound-guided fine needle aspiration is a method used to diagnose and grade pancreatic neuroendocrine neoplasms using the World Health Organization 2010 classification based on Ki67 labelling index. It is still unclear whether this method is adequate for grading leading to several recent empirical studies.
Objectives: This study aims to review the latest research regarding the utility of endoscopic ultrasound-guided fine needle aspiration in WHO grading of pancreatic neuroendocrine neoplasms by Ki67 labelling index.
Methods: A literature search for empirical studies from January 2014 to December 2015 with empirical data comparing WHO grading by Ki67 labelling index in endoscopic ultrasound-guided fine needle aspiration samples with those from matching surgical resections was carried out. The seven articles yielded were chosen for review.
Results: The papers varied in their quality of patient sampling and reporting of methods, and overall the rarity of pancreatic neuroendocrine neoplasms resulted in small patient numbers. Concordance between endoscopic ultrasound-guided fine needle aspiration and surgical resection grade varied from 57.6% to 89.5%, but was generally nearer the higher figure. Concordance may be improved by excluding large tumours, or altering the Ki67 labelling index cut-off for grades G1 and G2 pancreatic neuroendocrine neoplasms. Low cell-counts in endoscopic ultrasound-guided fine needle aspiration samples probably hinders accuracy.
Conclusion: Endoscopic ultrasound-guided fine needle aspiration has the ability to predict the grade of pancreatic neuroendocrine neoplasms according to the WHO classification with a modest but significant level of inaccuracy. However, clinicians should be wary of grades obtained by samples with low cell-counts or from larger tumours.

P60

Anaplastic Thyroid Carcinoma-a Clinicopathological Analysis

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Introduction: Anaplastic carcinoma of thyroid is an aggressive tumour which usually shows extra-thyroidal extension and regional metastases at the time of presentation. It represents more than half of deaths from all thyroid cancers with a mortality of about 90%.This study aimed to review clinicopathological features of anaplastic thyroid carcinomas presenting at a single centre.
Methods: Pathology database was searched for all cases of anaplastic thyroid carcinoma diagnosed between January 2010 and january 2016.Patient's age, gender, cytological assessment, specimen type, presence of pe-existing differentiated component, squamous cell carcinoma component, spindle cell areas, rhabdoid areas, epithelial growth, lymph node involvement and immunostaining results were noted
Results: Thirteen patients were diagnosed with anaplastic thyroid carcinoma during the study period. Patients' median age was 69 years (range 58-86) including 11 women and two men. Cytological diagnosis (FNAC) was available in six cases with following results; Thy1(1), Thy3(1), Thy4(1) and Thy5(3). Surgical specimen type included total thyroidectomy(n=6, 46%), lobectomy(n=4,31%) and incisional biopsy(n=3, 23%). Cervical lymph node specimens were available in 8 patients, of which lymph node metastases were seen in 5 (62.5%) cases. Pre-existing papillary thyroid carcinoma was seen in 2 patients and follicular neoplasm in 7 patients. Spindle cell areas were seen in 11/13 (85%) patients, Rhabdoid areas in 2/13 (15%), Epithelial growth in 7/13 (54%) and Squamous cell carcinoma component in 1/13(8%). Immunohistochemistry was performed in 11 cases. Most commonly observed antibodies were CK AE1/AE3 (n=6/11,55%), p53(n=1/12, 8%) and TTF-1 (n=4/11, 36%). Ki-67 was performed in one case only which showed 80% staining. Pathological stage was available in 9 cases;pT3 (4/9, 44%) and pT4(5/9, 56%).

P61

Evaluation of Thy3 Cytology: Reporting Rates, Sub-classification and Outcomes

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Introduction: The Royal College of Pathologists (RCPath) Thy3 thyroid cytology reporting guidelines (2009) recommended sub-classification into Thy3a and Thy3f. A local audit in 2009-10 showed a subclassification rate of 73% prompting changes for improved reporting. This study aims to review current performance and additionally reviews Thy3 outcomes.
Methods: Computer records were searched for all Thy3 cases reported between 25/07/2013 and 24/07/2015. All Thy3 reports were checked for sub-classification (Thy3a/Thy3f). Subsequent excision pathology results were compared for cytology/histology correlation.
Results: Of 1093 thyroid cytology cases reported in 2years, 86 were reported as Thy3 (7.86%). Further sub-classification was available in 85(99%) cases; Thy3f 50(58.14%), Thy3a 35(40.7%), and unclassified 1 case. This is significantly higher than the earlier reported 73% sub-classification rate (2009-10 audit). Matched surgical outcome was available in 61(70.93%) cases; Thy3a (20) and Thy3f (41). Surgical excisions showed follicular adenoma (28 cases, 46%), adenomatoid nodule (12 cases, 20%), follicular carcinoma (12 cases, 20%), hyperplasia/goitre(4 cases, 7%), atypical adenoma(2 cases, 3%), follicular variant of encysted papillary carcinoma(1 case), autoimmune thyroiditis (1case) and lymphocytic thyroiditis (1 case). Incidental malignancies included 1 papillary microcarcinoma with adenomatoid nodule, 2 papillary micorcarcinomas with follicular adenoma and 2 follicular variants of papillary microcarcinoma with follicular adenoma. Overall, on surgical resection, 44 cases (72%) showed neoplasia; Thy3a (11cases, 55%) and Th3f (32 cases, 78%). Malignancy was noted in 18 (29.5%) cases, viz. Follicular carcinoma 12(19.67%), Papillary carcinoma 6(9.83%). Positive Predictive Value (PPV) for neoplasia was Thy3a (55%), Thy3f (78%). PPV for malignancy was Thy3a (20%), Thy3f (32%)
Conclusion: Repeat audit has shown a significant improvement in Thy3 sub-classification.

P62

Primary Well Differentiated Neuroendocrine Tumour of the Thyroid Gland – Report of Case

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A case of primary well differentiated neuroendocrine tumour (PWDNET) of the thyroid gland in a 50 y old female patient without MEN syndrome is presented. Ultrasound showed an incidental 12mm hypoechoic left thyroid nodule, cytologically Thy3a, which on low power resembled an atypical thyroid follicular adenoma, but which immmunohistochemically was a low grade (Ki67<1%) calcitonin negative neuroendocrine tumour (NET) derived from follicular thyroid lineage; TTF1+ve, CD56 +ve, Chr +ve, Syn +ve, thyroglobulin –ve, HBME 1 –ve, mCEA –ve, S100 –ve & parathormone –ve. Primary thyroid NETs are rare. The majority of neuroendocrine derived primary thyroid tumours are medullary thyroid carcinomas (MTC) which are calcitonin & CEA +ve although calcitonin –ve MTCs are described. The differential diagnosis of PWDNET is metastastic NET to the thyroid from other sites, particularly gastropancreatic NETs and other primary thyroid lesions that may display neuroendocrine lineage such as intrathyroidal paraganglioma, calcitonin –ve MTC and poorly differentiated calcitonin-negative neuroendocrine tumours. Although rare, it is important to distinguish non-MTC neuroendocrine tumours of the thyroid from MTC's as the genetic implications, management and follow up may differ.

P63

Adrenal Lymphangioma Presenting as a Non Functional Adrenal Cyst

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Purpose of the Study: We describe a case of an adrenal lymphangioma which presented as a non-functioning adrenal cyst.
Case Report: A male patient presented with left scrotal pain in 2012. Ultrasound scan of the scrotum revealed a varicocele. Further upper genito-urinary tract imaging with CT scan showed a 5.5 cm left adrenal mass with calcification. On investigation adrenal biochemistry was within normal limits with plasma metanephrine levels of 0.20 nmol/l. Clinically and radiologically the features were those of a non-functional adrenal cyst. As the lesion was large (> 5 cm), a decision for excision was undertaken and the patient underwent a left adrenalectomy. Macroscopically, a 5.5 x 3.7 cm cyst was identified within the adrenal gland with a multiloculated cut surface and mucoid contents. Microscopically, thin septae formed the walls of the locules; these were filled with eosinophilic fluid and contained sheet of macrophages. Focal calcification was noted. The locules were lined by flattened endothelial cells which expressed CD31 but not CD34, CK7 or CK20, confirming a diagnosis of adrenal lymphangioma. The lesion had been completely excised and required no further treatment.
Conclusion: While non-functioning cysts of the adrenal gland are not uncommon in clinical practice (due to the ease with which asymptomatic, incidental lesions are picked up on imaging) we would like to point out that lymphangiomas can also present in the adrenal and clinically and radiologically are indistinguishable from a conventional non-functional adrenal cyst.

P64

A Unique Case of Benign Pleomorphic Adenoma Metastasizing to the Thyroid Gland and Liver

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Purpose of the Study: We describe the first reported metastasis from the parotid to the thyroid gland.
Case Report: We report a case of a 68-year-old lady who was initially investigated for a space-occupying lesion in the liver but was found to have metastases in the thyroid and liver as well as recurrence of the parotid primary. The clinical findings, management, and a review of the current literature are reported. Metastases from PSAs are extraordinary. We describe the first reported metastasis from the parotid to the thyroid gland.
Conclusion: In this case, we report an excellent outcome post-operatively and argue for the benefit of long-term follow-up.

P65

Low Grade Clear Cell Microcystic Adenoma of the Sinonasal Cavity: A Case Report

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We describe a primary clear cell microcystic adenoma of the sinonasal cavity in a patient with Von Hippel Lindau disease (VHL). The 39 year old patient had a molecularly-confirmed diagnosis of VHL-disease and had previous clear cell renal cell carcinoma (RCC) with resection/ablation over the past 10 years. He presented with epistaxis and was found to have an expansile mass in the ethmoid sinus and initial biopsy was reported as metastatic clear cell RCC. Full clinical staging showed no other metastatic disease. Tumour morphology demonstrated a tubulocystic pattern with abundant mucoid material and PAS-positive secretions within the cystic spaces. Sinonasal tumour cells had low grade nuclei, glycogen-rich cytoplasm and a common brush border. Immunohistochemistry revealed tumour CD10 and RCC negativity and positive staining for CK7, CK20 and epithelial marker AE1/3. Comparison with the previous clear cell RCC showed dissimilar phenotypes and consensus was reached that the tumour was a primary low grade clear cell microcystic adenoma. There has been one previous case of a primary low grade clear cell microcystic adenoma of the sinonasal cavity reported in a patient with VHL, with molecular analysis confirming tumour VHL-association. In the case we describe here, analysis of DNA extracted from tumour tissue showed no loss of the wild type allele at the *VHL* locus and did not support tumour association with VHL disease. It was not possible to look for a loss-of-function tumour mutation using dosage analysis, but gene sequencing showed that complete loss of heterozygosity (LOH) was unlikely due to heterozygosity for an intronic polymorphism. The association of primary low grade clear cell microcystic adenoma of the nasal cavity with VHL disease remains speculative. These lesions are benign but are likely to require regular surveillance and may require repeated surgical excision.

P66

Audit of the Dataset for Histopathology Reporting of Nodal Excisions and Neck Dissection Specimens Associated with Head and Neck Carcinoma

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Purpose of the Study: The RCPath introduced new datasets in 2013 for head and neck cancer reporting. The aims of this audit were to ascertain whether we were meeting the standards set and identify areas for improvement.
Standards: 95% of reports should have T, M and P codes 90% of cases discussed at MDT meetings should have pathology reports/core data available 80% of resection specimens include 100% data items presented in a structured format 80% of biopsies reported within 7 days 80% of all specimens reported within 10 days
Methods: A search was carried out to find all relevant cases from 1/11/13 to 31/10/14. 138 cases were found. The following information was collected: Patient age and sex Presence or absence of SNOMED T, M and P codes Was relevant clinical information provided? How many lymph nodes collected? Was a proforma used, and was all the necessary information recorded? Did the report include size of largest metastasis, type of tumour, and pTNM stage? Nature of specimen and turnaround time MDT meeting date
Results: 95 cases were included. Clinical information: Laterality present in 97%, anatomical levels 97% SNOMED codes: T code present in 100%, M code 99%, P code 92% MDT meetings: 87% reports ready for MDT, 10% not ready, 3% not known
Data items in structured format: proforma used in 75%, all data items present 78%
Specific information included: number of lymph nodes included in 99%, size of largest metastatic deposit included in 97%, tumour type included in 100%, pTNM stage included in 85%, Turnaround times: 80% of biopsies reported within 7 days, 84% of all specimens reported within 10 days
Conclusions: We met some of the standards, and failed to meet others.
Recommendations for Improvement: Educate surgeons that we need to know laterality and levels submitted; Record SNOMED P code; Record exact number of nodes present; Use a proforma; Add "size of largest metastasis" to the electronic proforma.

P67

Degenerative Nuclear Atypia in Pleomorphic Salivary Adenoma: Histological and Immunohistochemical Observations

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Purpose of the Study: To further understanding of cellular events in pleomorphic adenoma (PA) of salivary glands by investigating the occurrence and topographical distribution of nuclear changes regarded as degenerative.
Methods: A total of 84 cases of conventional PA were histologically diagnosed by this author over a 4-year period (34 males and 50 females; ages ranged from 21 to 83 years with a mean of 53.7 years; 62 located in the parotid, 7 in submandibular glands, 14 in minor glands and 1 in the neck). Several paraffin-embedded blocks of every case were extant. Haematoxylin and eosin-stained sections from them, were light-microscopically studied for unusual variations in size, shape and chromatin pattern of tumour-cell nuclei. Selected cases were examined by means of immunohistochemical techniques valuable in characterising cell phenotypes in PA, and cell cycle antigens.
Summary of Results: A single case (female, 26 years, palate; 1.2%) showed multiple, prominent cells with eosinophilic cytoplasm and bizarre, variably enlarged / giant, irregularly shaped and occasionally multi-vacuolated nuclei with variously condensed or stippled chromatin and no mitoses. These cells were variably dyscohesive and did not line lumina; were cytokeratin (CK) 5/6 (+, cytoplasmic), CK7 (+, cytoplasmic), CK14 (+, cytoplasmic), smooth muscle actin (+, cytoplasmic), p63 (+, nuclear), S-100 protein (+, nuclear and cytoplasmic), WT1 (+/-, cytoplasmic) and podoplanin (+/-, cytoplasmic); and did not stain for DOG1, p63, p16 or Ki67. The nuclear vacuoles were CK (+) and WT1 (+) - hence, interpreted as cytoplasmic inclusions.
Conclusions: Degenerative nuclear atypia in PA seems rare and associated with non-cycling cells of myomatous ('myoepithelial') and schwannomatous phenotype. The phenomenon does not seem related to malignant transformation. The particular phenotype of affected cells suggests similarities to the degenerative nuclear atypia in pleomorphic leiomyoma and ancient schwannoma.

P68

This abstract has been withdrawn

P69

The Effect of Systematic Fat Blocking on Lymph Node Yield in Upper Gastrointestinal Tract Resection Specimens

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Introduction: It has been shown that higher lymph node (LN) yields in upper gastrointestinal tract resection specimens are associated with improved patient outcomes. It is therefore incumbent on pathologists to take measures to maximise nodal yields from such specimens.
Objective: We sought to assess whether systematic fat blocking (SFB) combined with manual fat dissection (MFD) confers higher node yields than MFD alone.
Methods: Sixty resection specimens (45 oesophagogastrectomies and 15 total/subtotal gastrectomies) reported in one year were retrospectively reviewed. Specimens were divided into node retrieval by MFD only and by combined SFB and MFD, on the basis of whether extra blocks of fat were taken. Total numbers of nodes and numbers of involved nodes for these two groups were compared.
Results: Mean lymph node yield was 28.3 (3 to 94). SFB+MFD cases had a mean yield of 31.6, which was greater than 25.9 for MFD cases. Furthermore, SFB+MFD cases were associated with higher numbers of positive nodes (4.0) than MFD cases (3.3).
Conclusion: Our data show that nodal yield for upper gastrointestinal resection specimens at UHCW compares favourably with those at other large centres. We have also shown that SFB+MFD is associated with higher nodal yield than MFD alone. Given that high node yield has been shown to be associated with improved clinical outcomes, we propose that combining SFB with MFD may have an important role in routine histopathological practice.

P70

Colorectal Cancer Resection and Lymph Node Yield: Predictive Factors Through Specimen Report Analysis

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For accurate CRC staging, macroscopically the pathologist documents the tumour size (TS), clear margins, and lymph node yield (LNY) to inform management. Recovery of 12-15 LN without evidence of metastatic cancer achieves 80% negative predictive value (NPV). RCpath state a minimum average of 12 LN per specimen in the department. An initial literature review outlines factors that affect the LNY include gender, BMI, age, TS and stage, location and length of the specimen. This study aimed to establish correlation between specimen features and LNY that could inform recommendations.
305 specimen reports from 2012 and August 2014 were used. Resection site, dimensions and TS were investigated for correlation. The mesentery volume (MV) was crudely estimated using mesocolon height, specimen length and diameter. The correlation coefficient (CC) was calculated to explore predictive factors of LNY, (>0.5 considered to be a positive correlation). Complete data in these parameters totaled 192 reports. Patient age, tumour stage, and positive nodes were not reviewed.
LNY mean=23, median=21, range=1-87. There were weak positive correlations between LNY and specimen length (0.25), mesolcolic height (0.15) and MV (0.17). Dissector had an impact on total LNY. Mean LNY by a biomedical scientist = 28, specialist trainee=24, consultant=20. These differences were statistically significant (p<0/01).
Range in LN harvest implies multiple factors influence LNY. Greater average MV was found in RH, but same average LNY as LH, indicating lower node density in LH. TC yielded similar average MV, suggesting more MV in RH specimen. Higher CC values for length and diameter than volume suggests the LN are proximal to bowel rather than apex. Standardised measurements of these parameters may eliminate operator-related variation and contribute to a truer LNY. A larger sample size is would reduce confounding factors. The variation of dissector LNY is interesting and warrants further analysis.

P71

This abstract has been withdrawn

P72

Perforated Solitary Caecal Diverticulum in a Young Patient, Mimicking Acute Appendicitis Clinically and a Malignancy on Laparoscopy : A Case Report and Review of the Literature

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Introduction: Solitary caecal diverticulum is a benign and generally asymptomatic lesion which is rather uncommon. Most patients are young and present with right iliac fossa pain that is indistinguishable from acute appendicitis clinically. While perforation of the sigmoid diverticulum is common, perforation of the right sided colonic diverticulum is a very rare event. Most often, the correct diagnosis is discovered unexpectedly during surgical exploration for suspected appendicitis and a perforated caecal diverticulum can closely mimic a malignancy on laparoscopy. We describe a case of perforated solitary caecal diverticum in a young patient successfully treated with right hemicolectomy, discuss the clinicopathologic features and review the literature on this uncommon occurrence.
Case Description: A 19 year old male presented with a 24 hour history of pain in the right iliac fossa. He was mildly pyrexial, had localized abdominal tenderness and guarding. Full blood count was normal with a CRP of <5. Ultrasound abdomen showed an abscess in the right iliac fossa. An emergency laparoscopy for perforated appendicitis was performed which was later converted to open right hemicolectomy for caecal perforation. A right hemicolectomy specimen with part of omentum and a high tie lymph node was submitted. Macroscopically, the serosal surface of caecum was covered with purulent material. An abscess, measuring 30x35mm, with a small mucosa lined cyst in the centre identified, which raised the possibility of a diverticulum. Histological examination confirmed the presence of an inflamed diverticulum.
Discussion: A true solitary diverticulum of the right colon is usually of congenital type and affects younger age group. The disease has been reported to occur in 1-2% of surgical specimens in European and American series, as compare to 43-50% of specimens in Asian series, which may represent the hereditary differences. A preoperative diagnosis is important, but not always possible

P73

Sigmoid Colon Endometriosis with Regional Lymph Nodes Involvement: A Rare Cause of Bowel Obstruction Mimicking Carcinoma of Sigmoid Colon – A Case Report

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Introduction: Endometriosis involving serosal surface of sigmoid colon and rectum is not uncommon in child bearing age and is reported to occurs in 3 to 37 percent of patients with pelvic endometriosis. However, endometriosis presenting as intestinal obstruction is rare and occurs in less than 1% of cases. As there are no specific tests to make a preoperative diagnosis and due to similar colonoscopy and radiologic findings, differential diagnosis from colon cancer may be difficult. We report a case of sigmoid endometriosis in a 44-year-old woman presenting with abdominal mass and acute bowel obstruction.
Case Description: A 44-year-old, primiparous woman was admitted with a 2 days history of severe colicky abdominal pain, bilious vomiting and absolute constipation with no flatus. The patient had regular menses with LMP 3 weeks ago and no history of dyspareunia. Physical examination revealed a suspicious abdominal mass in left lower quadrant. Plain abdominal X-ray confirmed the large bowel obstruction. CT scan of abdomen and pelvis with contrast showed mural thickening with a 4cm mass in sigmoid colon, suggestive of a malignant process. Ultrasound (USG) showed a small uterine fibroid with a simple cyst in left ovary and a complex cyst (24x43x37cm) in right ovary. An uneventful Hartman's procedure was performed for suspicion of a colorectal carcinoma. Histopathological examination of the resected colon revealed deeply infiltrating, full thickness endometriosis in sigmoid colon with involvement of six regional lymph nodes and no evidence of malignancy. After eight months of primary surgery, patient underwent reversal of Hartman's procedure and total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH BSO), histology of which showed cervical and bilateral ovarian endometriosis.
Conclusion: Endometriosis is a rare cause of intestinal obstruction, often mistaken for malignancy and should be considered in differential diagnosis in a women of reproductive age.

P74

Auditing the Practice of a Healthcare Scientist in Training to Report Gastrointestinal Surgical Specimens

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Purpose of the Study: In 2012, a joint histopathology working group from the Royal College of Pathologists (RCPath) and Institute of Biomedical Science (IBMS) began a pilot study to train healthcare scientists in the reporting of gastrointestinal (GI) surgical specimens, which in 2015 became a full qualification. The training maps that of medical histopathology trainees, albeit to a much narrower breadth. At the end of stage C, after a minimum of 3 years, trainees sit an exit exam, set and marked at equivalent level to FRCPath part 2.
Methods: During stages A-C, number of patient requests reported, trainee diagnosis, final diagnosis and accuracy of diagnosis was audited. During stages B-C the consequences of any diagnostic errors was also audited.
Summary of Results: There was an annual increase in number of patient requests reported, consistent with gained experience and confidence. During each stage diagnostic accuracy increased (82.5% vs 91.4% vs 96.5%) whilst inaccuracy decreased (10.1% vs 3.9% vs 0.7%). There was a decrease in the severity of diagnostic errors made as experience developed (8.5% vs 2.9%). Most errors would have caused no or minimal harm. 9 diagnostic errors would have caused moderate harm. No diagnostic errors causing major harm were made in either stage of training.
Conclusions: Careful auditing of reporting accuracy and diagnostic errors is a useful tool for monitoring the progress of histopathology trainees. The decrease in incorrect diagnoses and diagnostic errors by stage C demonstrates the development of this trainee and demonstrates the ability of healthcare scientists to safely report gastrointestinal surgical specimens after a period of appropriate training. Healthcare scientists are a key part of the existing histopathology workforce. This development will allow them to become integrated members of the clinical histopathology team delivering a high quality service to patients.

P75

This abstract has been withdrawn

P76

BerEP4 and AE1/3 are Reliable Markers of Epithelial Content for Biomarker Discovery Using Reverse Phase Protein Arrays (RPPA)

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Background: Reverse phase protein array (RPPA) allows precise and high throughput quantification of protein. Homogenised samples are used thereby creating confounding data interpretation due to uncertainty around the precise cellular content of samples. We hypothesised that antibodies AE1/3 (targeting Type I/II keratins) and BerEP4 (targeting Epithelial Cell Adhesion Molecule) could be used to quantify epithelium in protein lysates from colorectal cancers (CRC).
Methods: Expression of AE1/3 and BerEP4 was quantified using RPPA and immunohistochemistry (IHC) in formalin-fixed paraffin embedded (FFPE) tissue samples from 19 cases of CRC. For RPPA, each sample was tested in sextuplicate. IHC was quantified by transforming images into stain-positive and stain-negative pixels using ImageJ.
Summary of Results: In RPPA, the intensity BerEP4 expression was greater than AE1/3 ($p<0.001$). There was excellent precision with a mean coefficient of variation of 5.9% for AE1/3 and 6.9% for BerEP4. Expression of AE1/3 and BerEP4 showed significant correlation ($r = 0.97$, $p <0.0001$). For IHC, both antibodies showed specific epithelial staining. There was no difference in intensity of expression but there were slight differences in extent of expression with, on average, 3.5% more AE1/3-positive pixels than BerEP4-positive pixels ($p=0.016$). The expression of the antibodies (number of positive pixels) showed significant correlation ($r = 0.95$, $p <0.0001$). Comparison of RPPA with IHC showed significant correlation for each antibody, (BerEP4: $r= 0.47$, $p=0.044$; AE1/3: $r=0.48$, $p = 0.037$). Combining the markers (using the geometric mean) improved the correlation between the assays ($r=0.50$, $p= 0.028$).
Conclusions: Expression of AE1/3 and BerEP4 correlate with each other in both RPPA and IHC. For each antibody, the two assays show significant correlation. Either antibody can be used to quantify tumour epithelium in RPPA although performance is improved if both markers are combine

P77

Immunohistochemical Characterisation of Histologically Normal and Inflamed Appendices in Patients with Right Iliac Fossa Pain

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Purpose of the Study: In the laparoscopic era opinion is divided about the removal of macroscopically normal appendices in patients presenting with right iliac fossa pain. We aimed to establish if subclinical inflammation was present in histologically normal vermiform appendices excised from such patients.
Methods: We studied four groups of patients: Group I (n=120) — uncomplicated acute appendicitis, Group II (n=118) — complicated appendicitis (perforation/gangrene), Group III (n=104) — histologically normal appendices excised for right iliac fossa pain and Group IV (n=106) — incidental appendicectomy at right colectomy (excluding inflammatory bowel disease). Immunohistochemistry was performed for IL-2R, IL-6 and TNF-a. The immunostaining was assessed quantitatively for IL-2R/TNF-a and semi-quantitatively for IL-6 in full-section specimens.
Summary of Results: Median, Q1-Q3 mucosal IL-2R expression in Groups I (46.6, 34.6-69.4), II (37.8, 25.4-60.4) and III (27.0, 20.2-42.4) was increased compared with Group IV (15.2, 7.9-25.0, p<0.01). Submucosal IL-2R expression in Group III (18.4, 10.1-34.7) was also increased compared with Group IV (2.8, 1.2-5.8, p<0.01). Epithelial IL-6 expression in Groups II (44.0, 8.0-97.0) and III (71.0, 18.5-130.0) was increased compared with Group I (9.0, 1.0-54.2, p<0.01) and Group IV (9.5, 1.0-63.0, p<0.01), as was IL-6 expression in non-epithelial cells in Group II (22.5, 10.6-46.0) and Group III (21.0, 10.0-42.2) compared with Group I (7.6, 3.0-17.3, p<0.01) and Group IV (8.3, 4.4-24.0, p<0.01). TNF-a expression was increased in Groups I (5.9, 3.2-9.9), II (6.8, 3.6-12.1) and III (9.8, 6.2-15.2) compared with Group IV (3.0, 1.4-4.7, p<0.01).
Conclusions: Histologically normal appendices from symptomatic patients exhibit pathological levels of cytokine expression suggesting the presence of an inflammatory process that cannot be detected on conventional microscopy.

P78

Epithelial Cell-derived a Disintegrin and Metalloproteinase-17 Confers Resistance to Colonic Inflammation Through EGFR Activation

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Epithelial regeneration is a key process for the recovery from ulcerative colitis (UC). Here we demonstrate that a disintegrin and metalloproteinase-17 (ADAM17), a main sheddase for tumour necrosis factor (TNF)-α, is essential for defensive epithelial properties against UC by promoting epithelial cell growth and goblet cell differentiation in mouse and human. Mice with systemic deletion of Adam17 developed severe dextran sulfate sodium-induced colitis when compared to mice with myeloid cell Adam17 deletion or control littermates. ADAM17 was predominantly expressed by regenerating epithelia in control mice, and its loss or inhibition attenuated epidermal growth factor receptor (EGFR) activation, epithelial proliferation, mucus production and barrier functions. Conversely, ectopic EGFR stimulation promoted epithelial regeneration thereby partially rescuing the severe colitis caused by ADAM17 deficiency. In UC patients, epithelial ADAM17 expression positively correlated with both cell proliferation and goblet cell number. These findings suggest that maintaining ADAM17-EGFR epithelial signaling is necessary for the recovery from UC and would be beneficial to therapeutic strategies targeting ADAM17-mediated TNF-α shedding.

P79

Genetic Mechanisms In Colorectal Polyposis

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Background: 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. Up to 90% of patients with a phenotype of typical FAP have a pathogenic APC germline mutation identified through sequencing of coding exons and deletion/duplication analysis via MLPA. Of those with a phenotype of attenuated FAP, an APC/MUTYH germline mutation is detected in only 10-50% of cases. The aim of this study is to identify novel constitutional mutations predisposing to multiple colorectal polyps (>10) when no mutation is identified (NMI) in APC/MUTYH during routine genetic diagnosis.
Methods: 60 patients with ≥10 colorectal polyps have been recruited. All are negative for APC/MUTYH mutations. Haloplex (Agilent) is being employed for targeted sequence capture of the genomic APC/MUTYH loci, and the ORFs of an additional 15 genes related to colorectal neoplasia. This is followed by ultradeep sequencing (UDS) on a HiSeq (Illumina). Samples undergo cDNA sequencing to screen for APC/MUTYH allelic imbalance (AI) and splicing abnormalities, and qPCR is employed to assess APC/MUTYH gene expression. Samples that remain NMI will undergo whole exome sequencing.
Results: * UDS has been completed on 31 patients. Putative novel pathogenic variants have been identified in APC, AXIN2 and POLE. * AI assays are in progress. So far 6/33 patients displayed AI in APC and no patients displayed AI in MUTYH. These findings are being confirmed with gene expression studies. * cDNA sequencing has been completed on 17 patients to date: there was no evidence of splicing abnormalities.
Conclusions: The identification of novel genetic variants/mechanisms responsible for colorectal polyposis may provide benefits for patients/families through improved diagnosis and clinical genetic management.
This work is supported by a PathSoc grant.

P80

Histopathology as the "Gold Standard"? Audit Study of the Certainty of Diagnosis in Duodenal Biopsies Taken to Investigate Patients for Coeliac Disease

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Coeliac disease is an autoimmune disease affecting the small bowel. It is triggered in genetically susceptible individuals by the ingestion of gluten, a family of proteins found in barley, rye, wheat and sometimes oats. Diagnosis usually relies on a combination of serology and histopathology, with histopathology being regarded as the "gold standard". Diagnosis is often complicated by self-imposed restriction of gluten intake (due to symptoms) and the lack of clear histopathological distinction between normal duodenum and duodenum with a low-level lymphocytic infiltrate. We aimed to quantify this diagnostic uncertainty in a retrospective audit study of the duodenal biopsies taken for suspected coeliac disease in our institution over a one year period.We identified 246 cases in which biopsies were taken because of a clinical suspicion of coeliac disease. Of these, we found that 11% (27) of patients referred with a suspicion of coeliac disease had "equivocal" biopsy results, while 8.5% (21) were reported as not showing changes of coeliac disease and 66% (163) were reported as consistent with or diagnostic of gluten sensitive enteropathy/ coeliac disease. This significant percentage of "equivocal" biopsy results suggests that there is significant room for improvement in coeliac disease diagnostics, for example by the development of an objective molecular test.

P81

Anatomy of the Transverse Colon and Possible Pathways of Aberrant Lymphatic Tumour Spread in Complete Mesocolic Excision for Colon Cancer

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Purpose of the Study: Lymph node metastases to the pancreatic and gastroepiploic nodes in transverse colon cancer have been described, however, the mode of spread in this area remains unclear. We aimed to describe the possible pathways of aberrant lymphatic spread in the area of the proximal superior mesenteric artery and vein, the greater omentum, and the lower pancreatic border.
Methods: Cadaveric dissections were undertaken in four donors according to the principles of complete mesocolic excision with central vascular ligation. The vascular anatomy of the transverse colon was scrutinized to determine possible pathways of lymphatic spread to the pancreatic and gastroepiploic lymph nodes.
Summary of Results: We identified vascular connections between the transverse colon and the greater omentum at the level of both the hepatic and the splenic flexures. Additionally, small vessels running from the transverse mesocolon to the lower pancreatic border in the area between the middle colic artery and the inferior mesenteric vein were demonstrated. Venous tributaries to the gastrocolic trunk could be exposed to highlight its surgical importance as a guiding structure in complete mesocolic excision for colon cancer.
Conclusions: We were able to confirm that it is feasible to clearly separate embryologic compartments by dissecting along predefined tissue planes when performing complete mesocolic excision. However, the close vicinity of the foregut, midgut, and hindgut results in vascular connections that might serve as potential pathways for lymphatic metastatic spread of transverse colon cancer. *Acknowledgements: The work is supported by a Pathsoc Career Development Fellowship.*

P82

CT Assessment of Right Colonic Arterial Anatomy before and after Colon Cancer Resection - A Potential Marker for Quality and Extent of Surgery?

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Purpose of the Study: The benefit of high ligation in surgery for right colon cancer has been the subject of much debate, with conflicting evidence as to the optimum extent of resection. The radicality of surgery is currently graded by pathologists based on analysis of the resected specimen. It is unknown whether computed tomography (CT) analysis of residual arterial stump length after surgery could be used as an alternative in vivo marker to determine the extent of mesenteric resection. Ileocolic artery stumps have been demonstrated previously on CT after right hemicolectomy, but only in the early postoperative period.
Methods: We undertook a retrospective analysis of the routine pre-operative staging and post-operative follow-up CT scans for 151 patients who had undergone surgery for right-sided colon cancer. The preoperative right colonic arterial anatomy and the postoperative arterial stumps were analyzed and measured.
Summary of Results: Preoperatively, identification of the ileocolic (98.8%), middle colic (94.7%), and right colic arteries (23.8%) was comparable to catheter angiogram studies. Postoperative ileocolic stumps were consistently identified (88.3%) many months (average, 2 years and 42 days) after surgical resection, and were significantly longer than expected for a standard D2 resection (mean 28.1 mm, range 2.5 to 74.3 mm). Mean arterial stump length was significantly longer than anticipated stump length ($p<0.001$).
Conclusions: This is the first study to successfully demonstrate ileocolic arterial stumps using routine portal venous CT many months after right colon cancer resection. Further prospective studies should assess whether arterial stumps can be used as an in vivo marker of surgical quality and radicality.
Acknowledgements: This work is supported by a Pathsoc Career Development Fellowship.

P83

Expression of PD-1 and CTLA-4 Ligands in Invasive Bladder Cancer Mouse Models

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Introduction: Immune therapies which target T-cell checkpoint blockade have recently emerged. There are two main T-cell checkpoint inhibitors: Programmed cell death receptor 1 (PD-1) and Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4). In order to overcome cell-mediated immunity, tumour cells express their ligands to inhibit T-cell activity. Their main ligands are Programmed Death Ligand 1 (PDL1) and Cluster of Differentiation 80 (CD80), respectively. Various mouse models of invasive bladder cancer have been developed, which include models with Fibroblast Growth Factor Receptor 3 (FGFR3) mutations and PTEN loss. Whether tumours developed in these models acquire T-cell checkpoint blockade is unknown.
Aims: This study aims to establish whether carcinogen-induced mouse models of invasive bladder cancer are suitable for testing of T-cell blockade therapy, and to determine whether this form of immunotherapy could be advantageous when combined with therapies targeting bladder cancer-specific mutations, such as those in FGFR3.
Methods: Immunohistochemistry using antibodies against PDL1 (ab58810, AbCam) and CD80 (sc-9091, Santa Cruz Biotechnology) was carried out. Samples were selected from urothelial tumours developed in N-butyl-N-(4-hydroxybutyl) nitrosamine (OH-BBN)-induced mouse lines which include: C57Bl6 (wild type), mice with FGFR3 mutations (S249C, K644E) and PTEN deletion.
Results: Positive staining of PDL1 and CD80 was observed in tumour cells and lymphocytes in some of the samples. PDL1 and CD80 staining was also observed in the urothelium and smooth muscle cells.
Conclusions: PDL1 and CD80 antibodies used were able to identify their respective antigenic targets in the mouse models in principle. The results provide scope for future research into T-cell checkpoint inhibition via PDL1 or CD80 combined with therapy targeting specific mutations.

P84

Screening for Anaerobes in Prostate Bacterial Flora of Prostate Cancer Patients in Urine Taken After Rectal Examination

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Purpose of the Study: Linkage between oxygen independent organisms and malignant transformation has been established with substantial evidence for H.pylori in stomach cancer and some, though substantially less data supporting Vitamin D deficiency as a promoting factor. Recent studies have linked another such organism, the Vitamin D responsive Propionibacterium.acnes (PA) with prostate cancer. Though such organisms have been associated in the past (Cooper et al), few have considered them causative. In an attempt to develop less invasive screening techniques for PA and other such organisms, we conducted a pilot study screening urine after rectal examination for the presence of anaerobes using MALDI-TOF for rapid bacterial identification.
Methods: Consecutive patients, predominantly on active surveillance, were recruited from a prostate cancer clinic. Control urine samples were surplus from routine screening of non-urological or renal conditions.
Results: 21 patients consented to examination of urine. 9 specimens (42.9%; 4 anaerobes, 3 micro-aerophilic & 1 facultive anaerobe) contained organisms identified growing optimally in a low concentration of oxygen. By contrast a limited sample of 10 non-prostate cancer urines yielded no anaerobes.
Conclusions: MALDI-TOF identification can be used to rapidly and reliably identify bacteria screen post prostate massage urine. It identified a higher proportion of organisms growing in a low concentration of oxygen than have been reported in previous studies using different techniques and in the small number of "normal" urines tested. Given increased knowledge about how micro-aerophilic organisms such as Helicobacter pylori induce stomach cancer, and the association of Streptoccocus gallolyticus with colon cancer it justifies larger studies in prostate cancer patients using MALDI-TOF to rapidly identify bacteria which have hitherto been problematic to identify.

P85

Does Separate Detrusor Muscle Sampling at Initial TURBT Improve the Adequacy of Pathological Staging and Clinical Outcomes in Patients with Invasive Grade 3 Urothelial Cell Carcinoma?

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Several studies have demonstrated poorer prognosis in patients with invasive high-grade (grade 3) urothelial cell carcinoma (UCC) undergoing transurethral resection of bladder tumours (TURBT) where no definitive microscopic detrusor muscle is present to assess for pT2 (muscle-invasive) disease. Separate detrusor muscle sampling at the time of TURBT has been introduced locally where clinical suspicion of invasion is high in order to improve the adequacy of pathological staging. In this study, the efficacy of this approach was evaluated.

Data were extracted from the electronic laboratory database for TURBT specimens over a 5 year period. We evaluated the presence of microscopic detrusor muscle presence according to whether detrusor muscle sampling had been performed. The data were stratified according to diagnosis including grade and stage if UCC was diagnosed. Invasive grade 3 UCC cases were selected for further analysis of specimen adequacy and clinical outcomes.

The study included 1601 specimens. Microscopic detrusor muscle presence was significantly higher in cases where detrusor muscle sampling was performed. We identified 394 invasive grade 3 UCCs, of which 216 were pT1 and 178 pT2. In 89 pT1 cases, no detrusor muscle was identified and so the staging was inadequate. Significantly fewer inadequate pT1 cases had had detrusor muscle sampling compared with those with an adequate pT1 or pT2 stage. Subsequent re-resections in those with initial inadequate pT1 stage showed that 40% were actually pT2 lesions. Those with inadequate staging at initial TURBT showed a small reduction in 48 month survival compared to adequately staged pT1 cases.

Separate detrusor muscle sampling at initial TURBT improves the ability of pathologists to stage invasive grade 3 UCCs adequately. Adequate initial pathological staging is important for prompt appropriate patient management and long-term outcomes. Our results support detrusor muscle sampling at TURBT to achieve this aim.

P86

Audit of Papillary Urothelial Bladder Tumours - a Look at Intradepartmental Grade Distribution and Compliance with the Dataset Produced by the Royal College of Pathologists

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Purpose of the Study: The latest dataset on the reporting of urothelial bladder cancers was published by the Royal College of Pathologists in 2013, and it outlined the core data items required in all pathology reports. The aims of our audit were to assess compliance by the four uropathologists who regularly report bladder biopsies in our department, analyse turnaround-time and the distribution of reported grades.

Methods: All transurethral resection of bladder tumour (TURB) specimens between December 2014 and October 2015 were reviewed, and a total of 106 cases were included in the audit that were diagnosed as papillary urothelial carcinomas. Compliance with the Royal College of Pathologists dataset was assessed by comparing the reports with the core data items.

Results: The overall average turnaround-time was 11.3 calendar days. Tumour stage and grade (both WHO 1973 and 2004) were reported in all 106 cases. The presence of the detrusor muscle was mentioned in 104/106 cases (98.1%), while lymphovascular invasion and associated carcinoma in situ were commented on in 32/106 (30.1%) and 95/106 (89.6%) cases respectively. Of all the reviewed cases, 64/106 (60.3%) were reported as pTa, and 42/106 (39.6%) were pT1 or pT2. Of the pTa cases, the distribution of cancer grades was as follows: grade 1 - 13/64 (20.3%), grade 2 low-grade - 16/64 (25.0%), grade 2 high-grade - 21/64 (32.8%), grade 3 - 14/64 (21.9%). The grading distribution by individual consultants was also examined.

Conclusions: Identifying underreported core data items is crucial to improving the quality of pathology reports and amending the departmental proforma to highlight these features could result in better reporting practice. Analysis of grade distribution could be extended to all departments in South Wales, to assess the differences between general and specialist centres.

P87

Ectopic Prostate Tissue in the Renal Pelvis

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Ectopic prostatic tissue occurs most commonly in the bladder and prostatic urethra and has been rarely reported in a variety of other sites but never in the upper urinary tract. We describe a unique case of ectopic prostatic tissue in the renal pelvis.

A 66 year old man presenting with rising serum PSA despite negative prostate biopsies underwent an MRI of the prostate gland that revealed a bulky tumour in the anterior prostate gland and an incidental polypoid tumour in the rectum that on excision was found to be a mixed adenoneuroendocrine tumour of intermediate grade malignancy. Subsequent staging CT scan of abdomen revealed a lesion in the left renal pelvis that on CT urogram corresponded to a 1.6cm filling defect, which was very suspicious for urothelial carcinoma. Rigid ureteroscopy revealed a slightly prominent infundibulopelvic angle but the overlying urothelium appeared normal. No obvious exophytic tumour was identified in the renal pelvis or calyces. Two cold cup biopsies of the renal pelvic angle were taken.

The biopsies showed normal surface urothelium with irregular branching glands in the submucosa. These glands showed typical morphology of benign prostate tissue with an inner layer of secretory cells and an outer layer of basal cells. On immunostaining, the basal cells were p63+ while the secretory cells were strongly positive for PSA, PSAP and NKX3.1 confirming the diagnosis of benign prostatic tissue. Possibility of specimen mix-up was excluded by comparing the biopsy genotype with that of his patient's abdominoperineal resection resection. As the renal pelvis has a different embryologic origin to the prostate, this ectopic prostatic tissue is unlikely to represent an embryogenic remnant.Radiological follow-up revealed no change in the lesion over 11 months. It is unclear whether the radiologically detected mass was due to ectopic prostatic tissue or whether this biopsy finding was merely coincidental.

P88

Xp11 Translocation Renal Cell Carcinoma with Morphologically and Immunophenotypically Variant Discrete Lymph Node Metastases

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Introduction: Xp11 translocation renal cell carcinoma (RCC), recently added to the WHO 2004 classification, is an uncommon tumour. These carcinomas have unusual and morphologically diverse appearances and can show nested solid growth, papillary architecture, tubular or trabecular patterns with spindled cells, clear cells, or cells with eosinophilic cytoplasm. We present a case of translocation-proven Xp11 RCC where resected lymph node metastases showed deposits with distinctly different morphologies and immunophenotypes correlating to different areas of the primary tumour.

Case Presentation: A 51 year old man underwent right nephrectomy and lymph node excision for a 52mm renal mass. Histology showed a tumour with heterogenous morphology including solid, tubular and papillary patterns with dispersed psammoma bodies. Different areas of the tumour contained clear cells or eosinophilic cells. Architecturally different zones of tumour displayed reciprocal immunophenotypes with tubular areas being diffusely AE1/AE3, CAM 5.2 and MNF116 positive, whilst adjacent papillary areas showed only very focal or negative staining. TFE3 rearrangement was detected by molecular cytogenetic studies, confirming the diagnosis. The tumour was advanced stage (pT3a pN1). Resected caval nodes contained discrete deposits with distinctly different morphologies corresponding to different areas of the primary tumour.

Discussion: This case illustrates that the range of morphological hetereogeneity seen in Xp11 translocation RCCs can possess different immunophenotypes and manifest as distinctly different metastases. The degree of morphological diversity can be diagnostically challenging. This is particularly important to remember when dealing with core biopsies because, depending on the area of the tumour or metastatic deposit sampled, the differential can include clear cell carcinoma (as in the pre-operative biopsy of this case) or papillary RCC.

P89

Use of Transperineal Template Biopsies of Prostate: A Review of one Pathology Department's Experience

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Purpose of the Study: To review our Department's experience of the use of transperineal (saturation) template biopsies (TPTB) of prostate for the first 14 months of its use at our institution, considering in particular the indication for TPTB and diagnostic yield.
Methods: We conducted a review of our files for TPTBs over a 14 month period from November 2014 to December 2015, inclusive. We reviewed the clinical information provided re the indication for biopsy, and MRI findings, and correlated this with the pathologic findings.
Results: 93 patients had TPTBs during this time. The mean number of cores per patient was 29 (range 13 to 51). Indications for TPTB were as follows: previous diagnosis of prostate cancer (PCA) with biochemical failure post-radiotherapy: 10.2%, repeat biopsy in patients on active surveillance (AS): 20.4%, persistently high or rising prostate-specific antigen with previously benign biopsies: 28.5%, repeat biopsy following a diagnosis of atypical small acinar proliferation (ASAP): 9.1%, previous transrectal biopsy (TRB) complicated by sepsis: 3%, MRI findings discrepant with prior biopsy: 20.4%, specific indication for TPTB vs. TRB uncertain: 8%. In patients without a prior diagnosis of PCA, a diagnosis of PCA was made in 24/53 (45%), ASAP in 5 (11%), high-grade prostatic intraepithelial neoplasian in 3 and benign in 21/53 (40%). Rate of PCA in TRB in our department averages 40% and of ASAP is <5%. In patients on AS, 12/20 (60%) had an increase in Gleason score to ≥7 on TPTB.
Conclusions: The rate of detection of PCA overall is not significantly higher in TPTB vs. TRB, (45% vs 40%) despite a highly selected group of patients. The rate of ASAP was higher in TPTB, suggesting the need for a subsequent TPTB. Given the significant additional work load generated by TPTB for the Pathology laboratory, rational evidence-based protocols for its use, particularly in conjunction with MRI findings, are warranted.

P90

Does Light Microscopic Dissection Improve the Adequacy of Renal Biopsies Submitted for Evaluation by Electron Microscopy?

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Purpose of the Study: Electron microscopy (EM) remains an important modality in the routine assessment of renal biopsies. Biopsies that do not include at least one glomerulus are considered inadequate for assessment. Historically our unit processed biopsies without prior assessment. A significant number of these biopsies were subsequently reported as being inadequate. In 2012 our practice changed and material was assessed with a dissecting microscope in order to isolate glomeruli prior to processing of biopsy material. We audited the number of cases that were reported as inadequate before and after this intervention.
Methods: All renal biopsies that were processed for EM were assessed for adequacy between the years 2009/10 and 2014/15. Adequacy was defined as the presence of at least one glomerulus. Comparison was made between those biopsies received before the change in our practice in 2012 and afterwards.
Results: The adequacy rate for renal EM has steadily improved from a low of 56% of cases in 2010/11 (135 of 242 biopsies) prior to routine light microscopic dissection, to a high of 80% of cases in 2014/15 (261 of 328 biopsies) after the routine use of the technique 2 in early 2012.
Conclusions: Routine evaluation of renal biopsies under the dissecting light microscope prior to processing improves the quality of the material submitted for renal EM by enabling the direct identification and isolation of glomeruli. The technique is cheap and relatively easy to perform. Inadequate biopsies result in diagnostic delay, causing frustration for clinicians and patients and wasting valuable laboratory resources and time. Renal biopsies are also associated with a low but real risk of complications. Techniques that maximise possibility of producing adequate tissue for assessment by EM are of merit.

P91

Is Histological Examination of Toluidine Sections Prepared for Electron Microscopy of Value in the Reporting of Medical Renal Biopsies?

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Semi-thin toluidine blue stained sections of resin embedded material are examined to select appropriate blocks for EM. Block selection may be undertaken by technical staff or histopathologists, depending on local practice. In our centre it has been the practice for this to be done by histopathologists and it has been our practice to report on the pathology seen on the toluidine blue sections. With the closure of many EM units in the UK the ability of the pathologist to examine the tol blue sections is greatly diminished.
Methods: 113 consecutive adult native renal biopsies from a single unit were assessed for the utility of histological assessment of the toluidine blue stained semi thin resin sections. The report for each was assessed to establish whether there were any of the following criteria of significance present: 1. A change in the overall glomerulosclerosis rate of >10%. 2. A change in the crescent rate in glomerulonephritis of any kind of >10%. 3. A change in the Oxford score. 4. An isolated finding seen only on the tol blue sections. 5. A feature seen on the tol blue sections specifically referred to in the report as contributing to diagnosis.
Results: There was a change in the overall percentage of sclerosed glomeruli in 10 cases (9%) 4 of 43 cases (10%) had a 10% or more change in the percentage crescents. No biopsy of IgAN had a change in Oxford score (of 20 cases). Isolated specific findings were seen in 2 cases (2%) No report specified appearances or findings other than these two specific lesions seen. Overall there was a contribution made by examining the tol blue sections in 16 cases (14%)
Conclusion: The results suggest that diagnostic findings present only on tol blue sections are a rarity but that significant information that may finesse grade or stage of disease may be present in around 15% of cases. We would recommend that where tol blue sections are available then they should be examined for features that may assist in diagnosis.

P92

mTOR and HER Family are Promising Biomarkers in Ovarian Cancer

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Background: The mammalian target of rapamycin complex 1(mTORC1) is a downstream of the PI3K/Akt pathway which affects cancer development. mTORC1 has many downstream signalling effectors that can enhance different cellular responses. This study aims to investigate the expression of mTORC1 and HER family (HER1-4) proteins using immunohistochemistry in 195 annotated ovarian carcinomas (OC) prepared as tissue microarray.
Results: The expression of mTORC1 was homogenously cytoplasmic in 71% of cases. 70% of the cases showed cytoplasmic and/or membranous expression to EGFR. The majority of the cases (83.4%) scored 0 Hercept score for HER2 expression while score 3+ was observed in 1.2 % only. HER3 expressed in 22% of cases and it was mainly in cytoplasm with some weak membranous. HER4 was expressed in the nucleus (58%), membrane (70%) and cytoplasm (15%). High expression of mTORC1 was associated with serous type (p=0.05). Cytoplasmic HER4 was associated with serous, mucinous and endometroid types, (p=0.003)mTORC1 was associated with HER3 positivity (p=0.008) and cytoplasmic HER4 high expression (p=0.006). In addition, HER family proteins showed some association with each other in which, EGFR was positively associated with HER2 and HER3 (p=0.025, p=0.031 respectively). Outcome analysis has revealed that high mTORC1 expression was weakly associated with shorter progression free survival (p=0.076). However, none of the HER family proteins showed association with survival.
Conclusion: Our ovarian series showed a high expression of mTORC1 which was associated with some HER family protein expression. A larger series of cases is need to assess the significance of HER2 positivity in OC.

P93

Prognostic Value of Bilateral Salpingo-oopherectomy and Lymphadenectomy for Staging of Early Uterine Leiomyosarcoma

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Introduction: Uterine leiomyosarcoma (ULMS) account for ~1% of uterine malignancies and ~30% of all uterine sarcomas. Diagnosis is usually not made before surgery as the presenting symptoms for leiomyoma and leiomyosarcoma overlap. Currently, the corner stone of treatment for leiomyosarcoma involves hysterectomy with bilateral salpingo-oopherectomy (BSO). In early stage leiomyosarcoma routine pelvic and para-aortic lymph node resection is not indicated, as incidence of lymph node metastasis is 3%. The incidence of occult ovarian involvement is low in early stages (3.4-3.9%) therefore ovarian preservation in premenopausal should be considered. This study aimed to assess the prognostic value of BSO and lymphadenectomy for staging of early primary ULMS.
Material and Methods: A series of ULMS (n=45) diagnosed between 1989-2015 at a tertiary centre was reviewed. Comprehensive data investigated include age, date of initial diagnosis, tumour histology, grade, stage, size, adjuvant therapies, and follow-up disease status and survival.
Results: The age range of the patients was 41-79 years. The tumour size ranged from 32-250mm. Nuclear grade was moderate to high in all cases except 3 cases. 94% of cases had spindle cell morphology with mitotic index ranging from 3-80/10 hpf. Necrosis was present in 95% while vascular invasion was present in 22%. Of these, 38/45 cases underwent BSO. 3/38 (7.8 %) cases showed ovarian involvement. Only 4 cases had lymph node resection during surgery and none showed metastatic disease at initial diagnosis. 6 cases had local recurrence and 9 cases distant metastasis to liver or lungs. 2/9 cases with distant metastases had additional bone or nodal disease. Most of the cases except one with local recurrence /metastasis had no ovarian involvement during initial diagnosis.
Conclusion: The incidence of ovarian and lymph node metastases in ULMS is very low. Ovarian preservation may be considered in premenopausal patients with early-stage ULMS.

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Endometrioid Adenocarcinoma Arising from Endometriosis of the Colon: A Report of 2 Cases

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Introduction: Malignant transformation of endometriosis occurs in 0.7–1% of patients and among extragonadal sites, the colorectum is involved in only 5% of endometriosis-associated malignant tumours. We describe two cases of endometrioid adenocarcinoma arising in endometriosis of the recto-sigmoid with a remote history of hysterectomy.
Case Reports: 1). A 68-year-old woman with a history of hysterectomy for endometriosis 19 years ago presented with an acute worsening of bowel symptoms. Investigations revealed large bowel obstruction due to a recto-sigmoid stricture. A low anterior resection was performed following a diagnosis of high grade dysplasia on biopsy. 2). A 72-year-old woman with a history of hysterectomy for endometrioid adenocarcinoma 15 years ago presented with a three-month history of changing bowel habit and rectal bleeding. CT scan showed a thickened sigmoid colon. Following an inconclusive biopsy, a sigmoid colectomy was performed. Both patients are disease-free following adjuvant chemotherapy.
Histology: Histopathological examination in both cases revealed grade 1 endometrioid adenocarcinoma with focal squamous differentiation involving the full thickness of the bowel wall. Both cases showed foci of endometriosis adjacent to tumour. Immunohistochemically in each case the tumour was positive for CK7, ER, and PR and negative for CDX2 and CK20.
Discussion: Endometrioid adenocarcinoma arising from endometriosis often mimics primary colonic adenocarcinoma and is diagnostically challenging. Our cases highlight the importance of clinical history (particularly of prior surgery and endometriosis), careful morphologic evaluation and immunohistochemistry in arriving at the correct diagnosis to allow optimal patient management.

P95

Uterine Leiomyomata with Metastatic Carcinoma – A Very Rare Finding

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Leiomyomata are common benign uterine tumours with an incidence of up to 70% in normal uteri. Leiomyosarcomas are rare. Metastatic carcinoma within leiomyomata is very rarely encountered. Here we present two cases of metastatic carcinoma within leiomyomata and review the associated literature.
Our first case was a 48 year old female who underwent hysterectomy for multiple "fibroids". Macroscopic examination showed one of the "fibroids" to contain an area of necrosis. Histologically this showed infiltration by mitotically active, atypical, epithelioid cells floating within vast amounts of extracellular mucin. This was only seen in some of the sections of an otherwise benign leiomyoma. Immunohistochemistry confirmed the histological interpretation of a metastasis of gastro-intestinal origin. This finding prompted further investigation and a primary mucinous adenocarcinoma was identified within the caecum.
The second was that of a 59 year old female presenting with postmenopausal bleeding who underwent hysterectomy for a large intramural "fibroid". Macroscopically the "fibroid" showed a central area of necrosis. Histologically the features were suggestive of a metastatic breast carcinoma. Review of the patient's past medical history revealed previous mastectomy for an invasive ductal carcinoma of breast.
Atypical fibroids grossly and histologically unusual leiomyomata can be problematic in their interpretation. Apart from an array of differential diagnoses within smooth muscle neoplasms, iatrogenic effects must also be considered. We draw attention here to the rare but occasionally encountered possibility of metastasis within leiomyomata. Whilst metastatic breast carcinoma within leiomyomata is recognised in the literature we were unable to find any record of metastatic colorectal carcinoma within leiomyomata.

P96

Prostanoid Metabolic Enzymes in Endometrial Cancer

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Purpose of the Study: Endometrial cancer (EC) is the commonest gynaecological cancer in the UK. Type I ECs are oestrogen sensitive, develop from premalignant hyperplasia and are low-medium grade. Type II ECs arise de novo, are high grade and have a worse prognosis. Given the role of prostaglandin-endoperoxide synthase (PTGS; cyclooxygenase) products prostaglandin (PG)F2 alpha and PGE2 in cancer, this study profiled them and their synthetic/catabolic enzymes in EC carcinogenesis.
Methods: PTGS1 and PTGS2 expression profiles were assessed by genome-wide expression microarray of laser capture microdissected endometrial specimens (n=81 normal, 30 hyperplastic, 118 cancerous). Matched tissue samples were analysed by mass spectrometry for PGF2 alpha, PGE2 and its inactive metabolite dihydro-15-keto PGE2 and normalised to protein. Tissue microarrays (n=419 ECs) were immunohistochemically stained for PTGS1, PTGS2 and the PG catabolic enzyme hydroxyprostaglandin dehydrogenase (HPGD).
Results: PTGS1 and HPGD were significantly underexpressed in hyperplasia and both cancer types ($p<0.05$). PTGS2 was significantly underexpressed in hyperplasia and type II cancers only ($p<0.05$). Immunohistochemistry reveals that, using a cut-off of 2.5, HPGD showed significantly stronger positivity in type I cancers, PTGS1 in type 2 cancers. Only HPGD had prognostic significance, where HPGD<2.5 predicted worse overall (log rank $p<0.01$) and progression free survival ($p<0.05$). Although PGE2 and PGF2 alpha concentrations were comparable across samples, dihydro 15-keto PGE2 levels were significantly lower in both cancer types.
Conclusions: Surprisingly, given their purported roles in carcinogenesis, levels of PGE2 and PGF2alpha; were not elevated in hyperplastic/neoplastic endometrium, which may reflect their function in endometrial physiology. However, the significantly decreased levels of dihydro 15-keto PGE2 in EC may reflect the decreased overall HPGD expression and, in turn, prostanoid turnover.

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Low Stage Uterine Serous Carcinoma: Does Reporting Lymphovascular Space Invasion Impact on Prognosis?

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Purpose of the Study: Endometrial serous carcinomas are Type II cancers which account for circa 5% of all endometrial carcinomas. Even in the absence of myometrial invasion, disseminated peritoneal spread is well-recognised. They follow an aggressive clinical course and carry an unfavourable prognosis. It remains unclear whether the presence of lymphovascular space invasion (LVSI) has any impact on outcome in low stage carcinomas (Stage Ia/b - invasion of less/more than half of the myometrium, respectively). This audit evaluated the merit of recognising LVSI in such cases.
Methods: All serous carcinomas (n=62) in our tertiary referral centre over the two year 2014-2015 period were reviewed and follow-up obtained.
Summary of Results: All patients survived. Twenty-seven (43.5%) serous carcinomas were Stage I; the remainder (35; 56.5%)were Stages II-IVb. Of the former, seven (25.9%) had histologically evident LVSI. Two recurrences (28.6%) were recorded in the LVSI group; five (25.0%) were seen in the group without LVSI (77-788 day range follow-up; median 424.5 and 406 days, respectively).
Conclusions: These findings suggest that there is little value in the histological recognition of LVSI in Stage I endometrial serous carcinoma as its presence does not provide any prognostic information.

P98

Carbonic Anhydrase IX Expression in Cervical Glandular Neoplasia and Pre-neoplasia

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Objective: The objectives of this study were to evaluate the immunohistochemical (IHC) expression of the hypoxia marker CAIX in endocervical adenocarcinoma and adenocarcinoma in situ (AIS) and to compare expression patterns of in situ and invasive disease.
Material and Methods: The archive of Galway University Hospital was searched from 2009-2014 and 65 LLETZ specimens with a diagnosis of AIS and/or invasive adenocarcinoma were identified. 21 cases were excluded from the study (slides not on file or no lesion remaining for IHC). Of the 44 cases included, 32 showed AIS alone, 10 showed invasive adenocarcinoma alone and 2 showed combined in situ and invasive disease. Invasive adenocarcinomas included 7 invasive endocervical adenocarcinoma,NOS, 1 clear cell, 1 serous, 3 villoglandular. Histology was reviewed and a representative block was selected. CAIX IHC was performed on a single representative section, on an automated platform (Ventana) using a mouse monoclonal antibody (clone TH22) at a dilution of 1/50. The CAIX expression was evaluated by 2 pathologists using a three tier scoring system (0-40% weak expression; 41-60% moderate expression; 61-100% strong expression).
Results: CAIX expression was seen in all 34 cases of AIS and was strong in 23 cases (68%), moderate in 3 cases (9%) and weak in 8 cases (23%) (Table 1). A total of 12 cases had an invasive component, 4(33 %) showed strong expression of CAIX . All were invasive endocervical adenocarcinoma, NOS. The remaining 8 (77%) showed weak CAIX expression. This included 3 villoglandular adenocarcinoma; 1 clear cell carcinoma, 1 serous carcinoma and 3 invasive endocervical adenocarcinoma, NOS. Of note, CAIX was negative in adjacent benign glands in all cases.
Conclusion: CAIX expression is common in endocervical glandular neoplasia and shows specific expression in neoplastic glands. CAIX is not useful in distinguishing in situ from invasive endocervical adenocarcinoma.

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Clinically Occult Serous Papillary Carcinoma of the Fallopian Tube Presenting in a Uterine Sample

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Purpose of the Study: .Case study of a 53 year old para 2 post menopausal female with a clinically occult serous papillary carcinoma of the Fallopian tube presenting in a uterine sample.
Methods and Results: A 53 year old para 2 post menopausal female presented after two episodes of post-menopausal bleeding. A pelvic ultrasound revealed an endometrial thickness of 6.8mm and a pipelle biopsy was performed. Histology revealed a papillary carcinoma raising the possibility of an endometrial intraepithelial carcinoma or a uterine serous carcinoma. The immunoprofile supported this diagnosis as tumour cells showed strong p53 expression. Surgical resection was undertaken and unexpectedly no tumour was noted in the endometrial cavity. The adnexae were then sampled extensively and one of the Fallopian tubes contained a 2mm focus of high-grade serous carcinoma surrounded by serous tubal intra-epithelial carcinoma. The immunophenotype was in keeping with serous differentiation as tumour cells were positive for ER, WT1 and p53. A diagnosis of Stage II C Fallopian tube adenocarcinoma (Grade 3 papillary) was made. The patient underwent adjuvant chemotherapy with curative intent. Four cycles of Carboplatin/Taxol were followed by two cycles of single agent Carboplatin. Genetic testing revealed a BRCA1 mutation, a variant of unknown significance. Currently, the patient remains in remission.
Conclusion: A number of malignant tumours arise in the female reproductive tract but Fallopian tube carcinoma is one of the most rare, comprising <1% of all malignancies (1). Prompt investigation and diagnosis are of importance to guide the correct clinical management and prevention of a potential misdiagnosis and inadequate surgical treatment.
References: (1) Hariprasad P, Hariprasad S, Srinivas T, Jayrama Shetty K. Primary Bilateral Fallopian Tube Carcinoma The Report of a Single Case with Review of the Literature. *Journal of Clinical and Diagnostic Research*. 2013 May 7 (5): 930-932.

P100

Disseminated Peritoneal Leiomyomatosis Associated with Endometriosis – Case Report and Review of the Literature

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Purpose of the Study: Disseminated peritoneal leiomyomatosis (DPL) is a rare condition characterised by the presence of multiple benign smooth muscle nodules throughout the peritoneal cavity. Very rarely, DPL has been reported in association with endometriosis. We present the ninth known case of DPL with endometriosis, review the literature, and discuss the possible aetiology of this very rare pathological finding.
Methods: A 50-year-old British woman with a family history of ovarian carcinoma underwent prophylactic laparoscopic bilateral salpingo-oopherectomy. During surgery, multiple pearly white nodules resembling disseminated malignancy were discovered over the peritoneal surfaces. Biopsies were taken for histopathological analysis.
Summary of Results: The biopsied lesions contained fibroadipose tissue with nodular areas of florid smooth muscle hyperplasia and foci of endometrial tissue. There was no evidence of malignancy. The endometrial components were not distinct from the smooth muscle nodules, but present within them. Thus, in places the lesions resembled miniature uterus-like masses. Immunohistochemical staining for Smooth Muscle Actin, CD10, oestrogen and progesterone receptors confirmed coexisting DPL and endometriosis.
Conclusions: We found several proposed aetiologies for DPL in the literature. Cases of DPL in patients with a history of surgical fibroid removal suggest possible iatrogenic engraftment of leiomyoma-debris onto the peritoneum. Others have noted associations of DPL with high oestrogen states, whilst a case series on a Caucasian family with DPL points to a role for genetic factors. In concert, DPL might occur in genetically susceptible individuals, who are exposed to oestrogen. Histologically, a population of submesothelial progenitor cells has been suggested as the origin of DPL. The rare coexistence of DPL with endometriosis makes a case for this theory, as these progenitors can form both smooth muscle and endometrial tissue.

P101

Primary Extrauterine Endometrial Stromal Sarcoma of the Peritoneum Arising in Endometriosis: A Case Report

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Introduction: Primary extrauterine endometrial stromal sarcoma (EESS) is a rare tumour and it is often associated with endometriosis. EESS may pose a diagnostic challenge especially when present in an extrauterine site with unusual histological features such as endometrioid-type glands.
Case Report: A 57 year old female with a history of irregular bleeding on HRT was found to have an endometrial polyp and right sided adnexal mass with peritoneal and uterine serosal deposits on a CT scan. Histology of US guided biopsies taken from the adnexal mass showed a low-grade ESS and the patient subsequently underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy. Multiple fluid filled cysts and circumscribed nodules on the serosal surface and parametria of the uterus and in the peritoneum were present.
Histology: Histology confirmed a low-grade ESS involving the peritoneum, serosal surface of the uterus, adnexae and parametrium with no evidence of tumour in the uterus. Endometrioid type glands within the tumour and foci of endometriosis away from the tumour were seen. The tumour was positive with WT1, CD10, ER, PR, SMA and negative for desmin, cyclin D1 and CD117. A diagnosis of a primary low grade EESS arising from endometriosis (FIGO stage IIC) was made. No recurrence was seen 7 months following resection.
Discussion: Malignant transformation occurs in 0.7% to 1% of cases of endometriosis and EESS may arise from foci of endometriosis. The treatment of choice for ESS is complete surgical removal. Chemotherapy and radiotherapy have not proven effective although hormonal therapy is considered of use.

P102

Are Histopathologists Overcalling Malignancy in Peritoneal Cytology and Abdominopelvic Biopsies?

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Purpose of the Study: Atypically proliferating serous tumours (APSTs) demonstrate characteristics of both benign and malignant tumours, and can complicate the diagnosis of gynaecological malignancy, based on the evidence of biopsy/cytology. This may induce the overcalling of APSTs as frank malignancy, leading to the selection of sub-optimal treatment, and psychological consequences associated with a cancer diagnosis. This audit aims to compare the results of diagnostic tests and final pathology for selected patients, and calculate the PPV for comparison with the 98% diagnostic accuracy rate, determined from the expected 1-2% error published by RCPath. This value will be used as the gold standard.
Methods: Patients discussed in Southampton Gynaecology Multidisciplinary Team meetings (MDTs) in 2013 and 2014 were reviewed. Patients who received both a suitable diagnostic test (pelvic mass/omental/peritoneal biopsy or ascitic fluid/peritoneal washing cytology) with a positive result, and a comparable final pathology were selected. Both test results were compared and the PPV was calculated. 52 patients were suitable for inclusion.
Summary of Results: The PPV was 96% (2sf); meaning 96% of patients with a positive diagnostic test result for a gynaecological cancer had their diagnosis confirmed on final pathology. This is 2% less than the identified comparator of 98%. However, the 95% confidence interval was 91-100% (2sf), meaning it is 95% certain that the true PPV of this sample lies in the range 91-100%.
Conclusions: As the 'gold standard' value is within the 95% confidence interval for the PPV, it is not possible to state that the diagnostic accuracy of abdominopelvic biopsy/peritoneal cytology is less than 98%. In conclusion, this audit has not shown that pathologists are making frequent errors in the diagnosis of gynaecological cancers; thus, the diagnostic pitfalls highlighted in the literature review do not seem to be inducing a large amount of overcalling of malignancy.

P103

An Interesting Case of Incidental Composite Follicular and In Situ Mantle Cell Lymphoma

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Introduction: Composite lymphoma (CL) is defined as the simultaneous presence of two morphologically and phenotypically different lymphomas in the same anatomic site. The incidence is unknown but it has been suggested that it accounts for 1-4% of all lymphomas. Cases of CL can pose diagnostic and therapeutic challenges.
Case Report: A 77 year old man with adenocarcinoma of the colon was noted to have cervical lymphadenopathy and three incidental lesions were found in the spleen. He underwent colonic resection and splenectomy with excision of the cervical node.
Histology and Ancillary Studies: Histological examination of the spleen and lymph node demonstrated partial effacement of the normal architecture by a follicular lymphoma (FL), grade 1-2. The lesion was CD20, CD10, BCL-2 E17(+) with a proliferation index of 40-50 %. There were also additional neoplastic nodules which showed a different histology and staining pattern. In these nodules the cells had centrocytic morphology and were CD20+, CD5+, IgD+, Cyclin D1 (patchy+) and SOX 11 (strongly+). FISH with break-apart probes detected the presence of a CCND1 translocation in the SOX-11(+) expanded nodules, but no translocation was present in the FL. In contrast BCL-6 translocation was found in the FL but not in the in-situ MCL. BCL-2 translocation was not identified.
Treatment and Follow-up: The patient was treated with FOLFOX chemotherapy (6 cycles) for his colonic cancer. The bone marrow was clear of CL and no rituximab or standard CHOP was prescribed for the lymhoma. On follow up he is free of disease with no further lymphadenopathies.
Discussion: The presence of a composite FL and mantle cell lymphoma is very rare, with only 7 previous cases being reported in the literature. Whether both components of CL share a common precursor cell or are unrelated is not yet clear. The rarity and complexity of composite lymphomas can present a diagnostic challenge and the ideal therapy in most cases remains to be determined.

P104

Synchronous Microscopic EBV (+) Diffuse Large B Cell Lymphoma of the Adrenal and Lymphoplasmacytic Lymphoma - de novo Disease or Transformation?

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Purpose of the Study: Case report. Malignant lymphomas primarily arising in the adrenal gland are very rare, accounting for less than 1% of all Non-Hodgkin's lymphomas1 and to our knowledge, there have been two case reports of an Ebstein Barr virus (EBV) (+) diffuse large B cell lymphoma (DLBCL) arising in an adrenal pseudocyst. We report a case of an incidental EBV (+) diffuse large B cell lymphoma (DLBCL) with an activated B cell phenotype arising in an adrenal pseudocyst in a 58-year-old male with a 7-year history of lymphoplasmacytic lymphoma (LPL). The EBV positive DLBCL was microscopic in size and present in the fibrinous exudate lining the pseudocyst while the EBV negative, low-grade lymphoplasmacytic lymphoma resided in the fibrous cyst wall. The patient underwent de-roofing of the same adrenal cyst 3 years prior to his current presentation; review of his previous adrenal histology revealed foci of lymphoplasmacytic lymphoma in the cyst wall, but not of DLBCL.
Conclusions: There have been reports of similar cases of microscopic EBV (+) diffuse large B cell lymphomas with an activated B cell phenotype, arising in enclosed cystic spaces2-5. However, in contrast to our case, in all these cases the DLBCL was an incidental extra-nodal primary tumour with no evidence of a pre-existing low-grade lymphoma. Since residual lymphoplasmacytic lymphoma was also present alongside the DLBCL and as both show similar light chain restriction, we propose that this case may represent transformation of a low to a high-grade lymphoma rather than a de novo primary EBV driven lymphoma as previously hypothesized.

P105

Determination of Histological Predictive Parameters for MYC Rearrangement in Diffuse Large B-cell Lymphoma

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Diffuse large B-cell lymphomas (DLBCL) are heterogeneous with varied clinical and molecular features and different prognoses. DLBCL stratification is becoming increasingly important for tailoring chemotherapy regimes. The majority of patients receive. combination immunochemotherapy: rituximab, cyclophosphamide, vincristine, doxorubicin and prednisolone (R-CHOP). The addition of cytarabine and methotrexate to R-CHOP (R-CODOX-M) improves outcome in cases with adverse prognosis. Fluorescence in situ hybridisation (FISH)-detected C-MYC translocation, with or without a concurrent translocation of BCL2 (a "double hit lymphoma"), is a poor prognostic features in DLBCL. FISH is a relatively expensive and time-consuming molecular test, making it important to identify good surrogate markers for groups at high risk of MYC rearrangement.Retrospective analysis of pathological parameters was conducted on 187 cases which had been sent for FISH, based on the previous selection criteria of a DLBCL with a proliferation index >80%. C-MYC translocation was identified in 30% of the cases. 18% of these cases were double hit lymphomas. CD10 expression was found to predict C-MYC translocation (P>0.0001) and concurrent C-MYC and BCl2 translocation (P=0.0076). IHC for C-MYC in >70% of the cells was also a strong predictor of C-MYC translocation (P=0.0038). However, a high proliferation index, the previously used selection criterion, was not. We derived new, evidence based criteria for FISH testing for potential double hit lymphomas: all DLBCL/ Burkitt's lymphoma (BL) overlap, all DLBCL of germinal centre type (using the Hans classifier) with >40% MYC+ by immunohistochemistry regardless of proliferation index.We are undertaking prospective analysis subsequent to the instigation of the new criteria, which may allow additional further refinement of our criteria for FISH studies.

P106

Significance of CXCL12-CXCR7 Signaling for CD20(-)CD138(-) Subpopulation in Lymphoplasmacytic Lymphoma

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Cancer cells with tumourigenic potential are limited to a small subpopulation known as cancer-initiating cells (CICs). Recently we investigated a candidate for CICs of lymphoplasmacytic lymphoma (LPL), which was positive for both B-cell marker CD20 and plasma-cell marker CD138. We reported that the subpopulation of CD20(-)CD138(-) phenotype, in which both markers were negative was a candidate for CICs of LPL using LPL cell line, MWCL-1. CICs are known to be plastic under stressed condition such as hypoxia, in which non-CICs are changed to CICs. In the present study, we investigated the plasticity to find candidates for advantageous microenvironments to CICs of LPL. The methods with MWCL-1 were as follows: culture in hypoxic (O_2: 1%) versus normoxic (O_2: 20%) conditions, microarray analysis, immunoblotting of CXCR7 or CXCR4, culture after addition of CXCL12, and assay for intracellular CXCL12 level. The methods with clinical samples of LPL were as follows: immunohistochemistry of CXCR7 and double staining of CD20 / CXCR7. We found that hypoxia induced the conversion of CD20(+)CD138(-) to CD20(-)CD138(-) phenotype when cells were cultured in hypoxia. We searched for markers preferentially expressed in CD20(-)CD138(-) subpopulation with microarray analysis and immunoblotting of CXCR7 or CXCR4, then the chemokine receptor CXCR7 was isolated. The number of CD20(-)CD138(-) cells increased in a time- and dose-dependent manner when cultured with CXCL12, a ligand of CXCR7. In addition, hypoxia enhanced the expression level of intracellular CXCL12 in MWCL-1. In clinical samples of LPL, a few tumour cells expressed CXCR7, in which CD20 expression was not detected.

We concluded that hypoxia and CXCL12-CXCR7 axis appeared to be advantageous microenvironments to CD20(-)CD138(-) cells. CXCR7 might be a favourable target for therapeutic intervention in some LPL cases.

P107

Can the Inter-Observer Agreement of Reporting Fibrosis in Medical Liver Core Biopsies be Improved with the Use of Reference Images? A Study Comparing Liver Subspecialists and General Histopathologists.

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Purpose of the Study: This study assesses if a set of reference images can improve inter-observer agreement in a simplified staging system of liver fibrosis, using four clinically relevant stages (none/early/bridging/late) that are non-numeric and independent of the type of chronic liver disease.

Method: In phase 1, eight subspecialist liver pathologists (raters) assessed the stage of liver fibrosis in 47 virtual slides stained with Sirius red, using the four categories above. The responses were collated, identifying those biopsies with either complete agreement or a 50:50 split across stages (17 in total). These were aligned, one per page, and used as reference images for typical threshold examples of fibrosis stage. In phase 2, a second set of 47 slides with similar range of stage was assessed by the raters, using the reference images as a guide. The same two sets of virtual slides were then assessed by a group of seven general pathologists, again using the reference images for phase 2.The results were analysed for all participants, and separately for the specialists and generalists. We identified the percentage agreement, the Kappa (K) scores and Krippendorff α for phase 1 and 2.

Summary of Results: Interim results show that data from all 15 participants found a modest improvement in overall agreement (66.7% to 70.5%), and eliminated cases spread over 3 stages in phase 2. The K and Krippendorff α results also confirm an improved agreement with the use of reference images with K rising from 0.55-0.60 and Krippendorff α from 0.55-0.57.When divided into the specialists and general pathologists, there was a similar improvement in % agreement from phase 1 to phase 2 along with the K and Krippendorff α for both cohorts.

Conclusion: Staging of liver fibrosis has inherent inter-observer variability. This study shows that the use of reference images results in an improved level of agreement, both among specialists and general histopathologists.

P108

Clinicopathological Assessment of Cholangiocarcinoma in a Tertiary Cancer Centre Over a Period of 4 years

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Introduction: Nearly 1000 people are diagnosed with bile duct cancer each year in Great Britain. Radical surgery remains the optimal therapy for cholangiocarcinoma, offering potential cure. The present study was conducted to assess the adverse prognostic factors associated with these cancers.

Methods: All operable cases of cholangiocarcinomas over a period of four years (2012-2015) in a tertiary cancer centre were identified on the Winpath histology database. Histology slides were reviewed and cases were classified on the basis of location into intrapancreatic, extra hepatic biliary tree and intrahepatic cancers. Various adverse prognostic factors were assessed. Clinical information wherever possible was obtained from medical records.

Results: Out of 41 cases reported as cholangiocarcinomas, 3 cases were excluded from the study (n=38). The male to female ratio was 4:1. 11 cases (29%) were in the extra hepatic biliary tree, 18 cases (47%) were intrapancreatic and 9 cases (24%) were intrahepatic in origin. The most common histology was grade 3 adenocarcinoma (29 cases). 14/18 (78%) intrapancreatic cholangiocarcinoma had lymph node metastasis and all 18 had higher stage (T3) at presentation. The overall mortality rate was 37 % (14/38 cases) and was slightly higher in advanced stage (pT3) intrapancreatic cancers (7/18 cases).

Conclusion: Intrapancreatic location of cholangiocarcinoma was most common in present study and a high mortality rate was associated with advanced stage and lymph node metastasis.

P109

Gall Bladder Carcinoma: A Clinicopathological Study of 25 Cases

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Introduction: Gall bladder carcinoma accounts for 0.3% of the all new cases in UK, being more common in females than males. The present study was conducted to assess the clinicopathological features of incidental and operable gall bladder carcinoma diagnosed in a tertiary cancer centre.
Materials and Methods: The study included all histologically proven gall bladder carcinoma cases between 01.10.2009 to 31.12.2015 on Winpath histology database. These include both the incidental carcinoma in cholecystectomy specimens and pre operatively diagnosed operable cases. However, inoperable cases were excluded from the study. Histology slides were reviewed and various prognostic parameters were analysed. Clinical information, wherever possible, was obtained from medical records.
Results: 25 cases of primary gall bladder carcinoma (including 5 referrals) were identified over a period of 6 years. Of these, 12 cases were incidental in patients with cholecystitis/cholelithiasis/benign polyp whereas 13 were operable cases of gall bladder/liver masses/malignant polyp. The M:F ratio was 1:2. Adenocarcinoma was the commonest histological subtype with poor differentiation seen in 12/25 cases (48%). 8 of 13 operable cases had advanced stage of disease pT3/pT4 (61.5%) and 7 of these died of disease. 4 of 12 incidental cases (pT2) had subsequent liver bed resection and did not show any residual disease.
Conclusion: Nearly 50% of the total gall bladder carcinoma cases in our study occurred incidentally in patients with cholecystitis/cholelithiasis (12/25 cases). Even when diagnosed pre-operatively, gall bladder carcinoma presented with advanced stage of disease and showed aggressive behaviour with poor prognosis.

P110

An Ex-Vivo Normothermic Porcine Pancreas Physiological Model and Implications for Islet cell Transplant

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Introduction: Total pancreatectomy with autologous islet transplantation is a recognised treatment for chronic pancreatitis. Its success depends in large part on the islet yield following isolation. We established an ex-vivo normothermic porcine pancreas perfusion model which is physiological and facilitates detailed study of both exocrine and endocrine function. This model can be used to study the early phase of islet isolation.
Methodology: Ten porcine pancreata were harvested and normothermically perfused with autologous blood. Serological parameters measured were: blood gas composition, routine biochemistry, glucose, insulin and glucagon levels. The volume of pancreatic juice secreted was also collected pre and post-secretin challenge. Histological staining with M30 cytoDEATH™ and Cleaved Caspase 3 antibodies.
Results: All pancreata were perfused for a median of 3 hours (range 2 – 4 hours) with a baseline perfusion pressure of 50mmHg, flow rate of 0.17 L.min-1 (range 0.14 – 0.33 L.min-1) and a blood lactate of 4.2mM (range 2.7mM – 8.4mM). All pancreata demonstrated cellular viability with evidence of oxygen consumption (arterio-venous O2 differential concentration) and produced pancreatic juice at a median rate of 3mL. hr-1 (range 2 – 17mL.hr-1). Pancreata demonstrated an increased insulin secretion following a glucose challenge. Immunohistochemical staining argued favourably for viabile pancreata at a cellular level.
Conclusion: This model avoids the use of live animals and can be used to investigate the islet yield in a porcine model following a period of machine perfusion. The physiological behaviour of this ex-vivo perfused pancreas model will allow the changes that occur in harvested pancreata and the potential effect they have on islet isolation, yield and viability to be studied in detail.

P111

A Two Year Review of Hepatobiliary and Pancreatic Frozen Sections; Accuracy, Second Opinions and Training Issues

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Purpose of the Study: Review of hepatobiliary and pancreatic frozen section reports assessing accuracy of diagnosis, the proportion reported by specialist GI pathologists, the proportion of second opinions, the effect of out of hours frozen sections and trainee involvement.
Methods: Reports were identified from the laboratory information system for assessment as above.
Results: A total of 121 reports contained sufficient detail for inclusion. Of these 108 (89.2%) showed complete agreement between frozen and paraffin sections. 91.7% of benign diagnoses and 95% of malignant diagnoses were confirmed as such. Of the 13 cases (10.8%) that did not show complete agreement 5 were initially described as suspicious and malignancy was subsequently confirmed. In 1 case a benign frozen section report was followed by a paraffin section report stating suspicious of malignancy. There were 4 cases where benign frozen section reports were followed by reports of malignancy but one of these was resolved in a subsequent frozen section at the same procedure. In 3 cases frozen section reports of malignancy were followed by paraffin section reports indicating a benign lesion and this was confirmed on review. A single consultant reported the frozen section(s) in 80.7% of cases, 2 consultants in 16.8% and 3 consultants in 2.5%. Discrepant cases were not significantly affected by greater consultant involvement. Only 3.3% of frozen sections were reported by non-specialists despite a general frozen section rota. Out of hours frozen sections (after 18.00) did not affect discrepancies. Trainee involvement was recorded in 29.8% of cases.
Conclusions: The study confirmed a high level of accuracy in frozen section reporting. Specialists were available for the majority of cases. Most cases did not have a second opinion but this had no significant impact on discrepancies. Trainees were exposed to training in this important area of histopathology.

P112

The Changing Name of a Rare Tumour; Intraductal Papillary Neoplasm of the Bile Duct

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We present a rare tumour of the bile duct which has seen a change in classification over the past ten years. Our patient is a 68 year old female who presented to her GP with long-standing dyspepsia. Initially referred for upper gastrointestinal endoscopy, further investigations revealed a biliary stricture and left hepatic atrophy. Cytology was not diagnostic and the patient underwent a partial hepatectomy, as the possibility of a cholangiocarcinoma could not be excluded. The histology revealed the stricture to be an intraductal papillary neoplasm of the bile duct (IPNB). These tumours are characterised by papillary growth within the bile duct lumen. They are regarded as a biliary counterpart of intraductal papillary mucinous neoplasm of the pancreas. This is a rare tumour which has seen its classification change over the years. IPNB was adopted in the 2010 World Health Organization (WHO) classification as a distinct clinical and pathological entity.

P113

PPAR Expression in Glioblastoma Has Prognostic Impact and is a Putative Glioma Stem Cell Predictive Biomarker

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Introduction: Primary glioblastomas have a 5% five year overall survival (OS). PPARγ and PPARα agonists have antineoplastic effects on *in vitro* and *in vivo* glioblastoma models. Our work seeks to establish the prognostic validity of PPAR expression in glioblastoma and assess PPAR druggability in glioblastoma stem cell (GSC) models.
Materials and Methods: The expression of PPARγ/α in 100 primary glioblastoma surgical samples and 10 control samples was assessed by IHC. The association between PPARγ/α expression and OS was analysed in the independent TCGA (n=538) database. PPARγ/α transcriptional levels were profiled by RT-qPCR. PPARγ/α expression was established in U87-MG and M059K human glioblastoma cell lines, G26 and G144 human GSC lines and 2 fetal neural stem cell lines. The effect and receptor dependency of pioglitazone and fenofibrate were evaluated *in vitro*.
Results: Our initial work has established that PPARγ/α is significantly overexpressed in primary glioblastoma samples compared to healthy living brain (IHC, western blot, RT-qPCR). Our survival analyses show that a mixed pattern of PPARγ/α expression is correlated with a significant increase in OS. This novel finding is confirmed by TCGA database analysis (p=0.008). Commercial glioblastoma cell lines and GSC can be inhibited using pioglitazone and fenofibrate. Pharmacological antagonism of PPARγ with GW9662 and PPARα with GW6471 does not rescue PPAR agonist-mediated cell death. Initial results suggest PPARγ/α are overexpressed in GSC compared to fetal neural stem cell lines.
Conclusions: We present preliminary evidence of PPARγ/α as novel prognostic biomarkers in glioblastoma. PPARγ/α may also be actionable predictive biomarkers, allowing selective targeting of glioblastoma/GSC, with minimal toxic effects to healthy brain. Our current work is focussed on shRNA mediated PPAR knockdown in GSC models.
Acknowledgements: Pathological Society and Jean Shanks Foundation Research Training Fellowship (HH).

P114

Histological Study of Lipids and Cholesterol in Alzheimer's Disease

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Alzheimer's disease (AD) is the most common dementia worldwide. Apart from age, the major risk factor for AD is the possession of the apolipoprotein E (APOE) ε4 allele; a lipid transport molecule. Genetic studies have suggested an association between cholesterol metabolism and AD pathogenesis. We hypothesise that lipid accumulation in the brain is important in the pathogenesis of AD. This study aims to elucidate the histological relationship of cholesterol/lipids in human AD.We examined the expression of cholesterol and lipids in 30 AD cases and 30 age-matched controls, obtained from the South West Dementia Brain Bank. Novel immunohistochemical techniques were used to label cholesterol and oxidised LDL (OxLDL), whilst traditional tinctorial methods e.g. Oil Red O (ORO) were used to assess neutral lipids, fatty esters and triglycerides. Digital images for each staining were obtained and quantified with ImageJ to obtain the load (%). A semi-quantitative analysis was performed to ascertain the pattern and intensity of ORO and cholesterol staining. No difference was seen in expression levels of ORO (p=0.567), cholesterol (p 0.723) or OxLDL (p=0.564) between cohorts, despite adjusting for the APOE genotype in both cholesterol (p=0.175) and OxLDL (p=0.686). ORO displayed a more prominent neuronal staining pattern in both cohorts compared to vascular staining (control: p=0.020; AD: p=0.004), whilst cholesterol demonstrated a more cellular pattern (control: p=0.002; AD: p<0.001). OxLDL only demonstrated a neuronal staining pattern. Correlations between cholesterol and OxLDL loads showed a negative association in the control group (r=-0.417; p=0.022) and a positive one in the AD group (r=0.415; p=0.023), potentially highlighting a different pathophysiology in AD. Despite these findings, further research is required to fully expose the elusive and complex role of lipids underlying the pathogenesis of AD.

P115

A Retrospective Audit Comparing Diagnosis and Trojani Grading of Sarcoma Core Biopsy Vs Resection Specimen.

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Purpose of the Study: This is a retrospective audit of sarcoma specimens reported in our institution. The aim is to assess the diagnostic accuracy of sarcoma core biopsy as compared to the surgical resection diagnosis. The predictive value of Trojani grading on the core biopsy as compared to the resection is also assessed.
Methods: Cases were reviewed using the central sarcoma data base and diagnoses/grading taken from the Winpath system. We performed a Chi-squared test (as the data are non-parametric) on 45/80 sarcoma cases (those which had complete grades given on core and resection) to assess for statistical significance in grading.
Summary of Results: Correlation of the core with resection was as follows: Malignant: Leiomyosarcoma (87%), Chondrosarcoma (100%), dedifferentiated liposarcoma (87%), undifferentiated sarcoma (54%). Intermediate: Desmoid type fibromatosis (100%), Dermatofibrosarcoma protuberans (100%), Solitary fibrous tumour (100%). Benign: Intra-muscular myxoma (100%), Schwannoma (100%). Trojani grading on core versus resection: Average overall Trojani grade on core: 2.11 and on resection: 2.64 (p= 0.0016). Average tumour differentiation score on core: 2.60 and on resection: 2.89 (p = 0.0019). Average mitotic count score on core: 1.49 and on resection: 2.18, (p = 0.0015). Average necrosis score on core: 0.60 and on resection: 0.96 (p = 0.0011).
Conclusions: For benign and intermediate tumour groups, there was 100% correlation between diagnosis on biopsy and resection. Correlation was poorest for undifferentiated sarcoma with a wider range of diagnoses given when the entire tumour morphology is seen on resection. The difference in Trojani grading was significant for all parameters assessed. This is likely due to the limited amount of tissue available on core leading to lower grading. Clinicians should be aware that the tumour subtyping/grade may change from the core biopsy impression, a factor which may influence patient management.

P116

Liposarcoma in an Apparently Benign Giant Oesophageal Polyp Showing MDM2 Amplification

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Purpose of the Study: Giant pedunculated fibrovascular polyps of the oesophagus are uncommon and may cause life-threatening asphyxia. Liposarcomas arising within these polyps are even more infrequent. Well-differentiated liposarcoma frequently shows amplification of MDM2 and CKD4 genes. We describe the case of a well-differentiated liposarcoma arising within an oesophageal polyp in a young woman that showed entirely benign morphology. However, amplification of MDM2 was identified by fluorescent in-situ hybridisation. We aimed to examine the frequency of cytogenetic alterations in the published literature in these unusual neoplasms.
Methods: We performed a literature search using Medline with the search terms OESOPHAGUS or OESOPHAGEAL and SARCOMA or LIPOSARCOMA or POLYP. We then hand-searched reference lists for all cases of liposarcoma arising within oesophageal polyps since 1950. We included all those with an adequate histological description.
Summary of Results: 22 cases were identified of which 16 were well-differentiated, 2 were de-differentiated, 2 were myxoid and 2 showed rhabdomyomatous differentiation. Amplification of MDM2 was assessed in 9 and CDK4 in 7 of the tumours, most commonly by immunohistochemistry. In all previously reported cases, there were morphological features of malignancy. One recurrence was reported.
Conclusions: Well-differentiated liposarcoma may be indistinguishable from a benign fibrovascular polyps of the oesophagus. It may be overlooked unless assessment for amplification of chromosome 12 regions including the MDM2 and CKD4 genes is performed.

P117

Metastatic Malignant Melanoma with Heterogeneous Neuroendocrine Differentiation – A Case Report

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Introduction: Malignant melanoma with neuroendocrine differentiation is a rare entity with only a few cases reported in the literature. We present a case of malignant melanoma with heterogeneous neuroendocrine differentiation that was misdiagnosed as high grade neuroendocrine carcinoma on a lymph node biopsy.
Case description: A 52 years old male was diagnosed with a primary malignant melanoma of nodular type of his left knee at the age of 34. 18 years later he presented with a rapidly growing left groin lymph node. A lymph node biopsy was performed showing large cohesive atypical cells in a nested pattern negative for all melanocytic markers, strongly positive for CD56 and focally positive for CAM5.2. The diagnosis of high grade neuroendocrine carcinoma was given at that time. One month later he had an excision of a lesion of his left thigh along with left groin dissection. The lesion on his left thigh was diagnosed as a nodular malignant melanoma positive for all melanocytic markers and negative for CD56. 3 out of 7 lymph nodes were positive for metastasis. The tumour cells within the lymph node showed heterogeneous immunoprofile with one showing positive staining for S100 and focal positive staining for CD56 and the other showing negative staining for all melanoma markers and positive staining for CD56 and CAM5.2. Two lymph nodes were sent for BRAF mutation and both were negative.
Discussion: This is the first case report of metastatic malignant melanoma with heterogeneous neuroendocrine differentiation. Previous cases have been reported showing expression of both melanocytic and neuroendocrine markers on both the primary tumour and the metastatic lymph nodes. The awareness of this entity will allow pathologists to consider this pathological lesion when coming across a metastatic lymph node of a patient with prior history of malignant melanoma that is positive for n.

P118

Skin Punch Biopsies – How Many Levels is enough?

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In dermatopathology, efficient laboratory processing of small skin specimen is paramount in providing a diagnosis in a timely manner. According to local guidelines, in processing skin punch biopsies, six levels are cut as standard to make a diagnosis. At times, the diagnosis is straight forward and these additional levels are not necessary but at other times further levels are requested. The purpose of this study is to determine whether the current local protocol of providing six levels is justified and which conditions require further levels to answer the clinical query. We looked at 153 skin punch biopsies in a prospective study over a period of 6 week and categorised them into those that required less than the standard 6 levels, those that were diagnosed based on the number of levels that were given and those that required more levels. We also looked at the pattern, to determine which clinical query required further level. Of the 153 biopsies, 47 (30.7%) were diagnosed within the first three levels, 71(40.1%) required the 6 levels and 35 (22.9%) required further levels. Of the biopsies that required further levels, 22 (62.8%) were for cancer, 1 (2.8%) for inflammatory conditions and 12 (34.2%) for other conditions. In conclusion, as nearly half of the specimen required 6 levels, our current local practise is justified. Of the biopsies that required further levels, over sixty percent were for cancer cases, again highlighting the importance of doing these further levels. Every time levels are requested, it increases the workload of that case, however, according to recent royal college guidelines on workload points allocation for dermatopathology, extra levels are not awarded additional points despite them being necessary. Based on this study we propose the review of these guidelines to allow for points to be allocated for extra levels to reflect their contribution to the workload for pathologists.

P119

Prognostic Factors in Thin Cutaneous Malignant Melanoma

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Introduction: The incidence of thin melanoma is rising, and melanoma is refractory to current therapy once metastases have occurred. However, prognosis for thin melanoma is extremely good. Hence, substantial attention has been given in the world literature to the exploration of patients with thin primary cutaneous malignant melanoma (CMM).
Purpose of the Study: To identify clinical, histopathological or molecular factors that may help in predicting disease progression and ultimately clinical outcome for subjects with thin CMM.
Methods: This was a cohort study conducted in the setting of an academic medical centre. 1265 subjects diagnosed between 2008 to 2015 were recruited. Of which, 463 were cases of thin CMM. Retrospective review of medical records was performed. The main study end point was OS for the thin CMM patient group, calculated from the date of diagnosis for primary melanoma to the date of death and censored accordingly to the date of last follow-up in living patients; death (n=21) was factored as a result of all causes. OS curves were estimated by using the Kaplan-Meier method. Univariate and multivariate survival analysis were performed using Cox's regression.
Summary of Results: Median follow-up for thin CMM subjects was 40 months (IQR 20 – 67 months). On univariate analysis, age HR 1.13 (95% CI: 1.08, 1.18), absent MR, brisk MR HR 7.31 (95% CI: 1.66, 32.18), extensive regression HR 3.73 (95% CI: 1.25, 11.12) and PI HR 50.07 (95% CI: 6.25, 401.09) were found to be significantly associated to OS ($p < 0.05$). On multivariate analysis, only age HR 1.14 (95% CI 1.08, 1.20) and extensive regression HR 4.61 (95% CI: 1.42, 14.97) remained significant ($p < 0.05$).
Limitations: Potential selection bias due to study design.
Conclusion: Age, regression and currently unknown factors will probably refine the melanoma stage-specific prognoses and treatment for individual patients. Histopathological prognostic markers still play a significant role.

P120

Expression of PD1 in Cutaneous Malignant Melanoma and its Prognostic Significance

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Introduction: PD-1 is a T cell co-receptor that has a major role in maintaining an immunosuppressive tumour microenvironment by working with its ligands PD-L1 and PD-L2. However, is PD-1 a good prognostic marker as it is a predictive marker for melanoma?
Purpose of the Study: To identify if PD-1 expression would help in predicting disease progression and ultimately clinical outcome for subjects with melanoma.
Methods: This was a retrospective cohort study conducted in the setting of an academic medical centre. 1265 subjects diagnosed between 2008 to 2015 were recruited. Of which 67 patients were selected for PD-1 immunostaining. Univariate survival analysis was performed using Cox's regression. In addition, the x2 test was used to assess significant differences in PD-1 expression between the cases and controls, while both the x2 or Kruskal-Wallis test was used to determine significant differences between PD-1 expression and clinicohistopathological parameters.
Summary of Results: Overall, PD-1 expression in both TILs and melanoma tumour cells was raised in subject metastases when compared to controls. On univariate analysis, PD-1 expression in both TILs HR 0.997 (95% CI, 0.990 - 1.003) and melanoma tumour cells HR 0.993 (95% CI, 0.984 - 1.002) displayed a weakly positive association to MFS surprisingly, however neither TIL nor melanoma tumour cell expression of PD-1 proved to be significantly associated to MFS. Moreover, PD-1 expression in TILs was significantly associated to the histopathological parameters histologic subtype and TILs. Interestingly, absent TILs on primary histological examination had a median PD-1 expression of 30 (IQR 3.75 - 112.5). All other parameters were not significantly different. PD-1 expression on melanoma tumour cells were insignificant for all clinical and histopathological parameters assessed.
Conclusion: PD-1 and currently unknown factors will probably refine the melanoma stage-specific prognoses and treatment for individual patients.

P121

Breslow Density as an Independent Prognostic Indicator in Cutaneous Malignant Melanoma

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Introduction: In 1970, Breslow thickness (BT) was shown to correlate with risk of metastasis and has since become a robust evidence-based data set item and T stage parameter. It seems reasonable to suppose that of two melanomas with the same BT, the one with fewer invasive cells and therefore lower density will have a better prognosis.
Objectives: We tested the hypothesis that a new variable, the Breslow density (BD), is a prognostic factor. The primary objective was to test whether its prognostic value was independent of BT.
Methods: 100 sequential cases of primary cutaneous malignant melanomas submitted since the 1st of January 2004 from the pathology archives of a University Hospital NHS Trust were selected. The cases had a median follow up of 87 months. The deepest invasive area was identified and a vertical window, limited inferiorly by the Breslow thickness, laterally by the x10 field diameter and superficially by the epidermis was selected such that the window contained the deepest cells and maximised the percentage of melanoma cells. An estimate of this percentage comprised the BD.
Results: BD showed almost perfect interobserver agreement (ICC 0.96, n=20). BD and BT showed significant correlation (p<0.001). Kaplan Meier survival estimates showed significant association between BD and metastasis free survival (MFS), melanoma specific survival (MSS) and overall survival (OS) (p=0.001, 0.0005 and 0.00009 respectively). BD hazard ratio (HR) using Cox proportional hazards regression showed univariate significance for MFS, MSS and OS respectively: HR 1.4 (1.02-1.07), p=0.03; 1.05 (1.02, 1.09), p=0.04; and 1.04 (1.02-1.06), p=0.0002. This effect remained significant when BT was added to the model, indicating that BD has independent prognostic value.
Conclusion: We show proof of concept that the measurement of BT has the potential to be enhanced by the addition of a density value, introducing the term Breslow density and paving the way for larger scale work.

P122

Regression and its Significance in Cutaneous Melanoma

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Introduction and Aims: Regression, and closely linked tumour infiltrating lymphocytes (TILs), are prognostic features recognised by the Royal College of Pathologists. However, literature is inconclusive about their prognostic significance. The confusion may be exacerbated by interpretive aspects of the guidelines. The study aimed to increase understanding of the role of regression, create a reproducible scoring system, and to assess whether scoring varies between UK centres.
Hypothesis: Regression scoring can be optimised and the optimised score will be predictive of outcome.
Methods: A standard operating procedure for grading regression within melanomas was developed. Agreement scores were collected for the method. Features of regression were assessed by light microscopy on 441 melanomas with metastasis-free, melanoma specific and overall survival follow up.
Results: On testing the scoring tool, a kappa agreement score 0.78 for regression was achieved, and an ICC of 0.81 for early regression and 0.98 for late regression. Preliminary analyses on a subset of 441cases showed regression in 89.6% of cases. Univariate Cox proportional hazard analysis showed a hazard ratio of 0.16 (CI 0.09-0.29, p=<0.01) in tumours exhibiting >40% regression, compared to the indicator of 0-5% regression. In multivariate analysis, the hazard ratio for the >40% regression group was 0.36 (0.18-0.71, p=<0.01), using 0-5% regression as the indicator.
Discussion and conclusions: Preliminary results suggest that regression scoring is valuable, as regression is shown in statistical analysis to have an effect on overall survival. With immune checkpoint inhibitor therapy, assessment of immune factors in melanoma may eventually have therapeutic impact.
Keywords: Regression, melanoma, tumour infiltrating lymphocytes

P123

An Audit on Compliance with the New Royal College of Pathologists Cutaneous Squamous Cell Carcinoma Dataset

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Purpose of the Study: To evaluate compliance with the revised cutaneous squamous cell carcinoma minimum reporting dataset from the Royal College of Pathologists.
Methods: Data were collected retrospectively for 50 patients using a local trust reporting system between the 1/9/2014 and 31/10/2014. Each report was scrutinized to identify whether each of the minimum dataset items were included. Results were discussed in the local audit meeting, individuals were provided with their personal results and teaching was targeted at new staff. A re-audit using the same methods and sample size was undertaken between 1/8/2015 and 31/8/2015.
Summary of Results: All cancer resections should be reported using core data items from the and the Royal College of Pathologists expects that this will occur 95% of the time. This study found only 42% compliance within our department. Of the 50 cases assessed 29 of them completely complied with the dataset. There were 21 reports which did not comply; 20 cases omitted one core item and one case omitted three core items. Fifteen cases did not specify a sub-type of squamous cell carcinoma, one case had an incorrect stage, five cases were not staged at all, two cases did not comment on peri-neural invasion and one did not report the macroscopic measurement of the lesion.The re-audit showed a vast improvement with 95% compliance. The only omissions of core dataset items being one case missing a subtype of squamous cell carcinoma, two cases did not include a macroscopic measurement of the tumour and two cases omitted the stage of the tumour but both of the later two cases were rectified with supplementary reports.
Conclusions: An audit to assess the adherence to the new minimum dataset for cutaneous squamous cell carcinoma reporting from the Royal College of Pathologists demonstrated a significant problem with ommisisons of core items. Following dissemination of results and teaching of new staff a re-audit demonstrated a significant improvement in compliance.

P124

A Retrospective Analysis of Skin Biopsies in Erythroderma - What Factors Help in Establishing the Diagnosis?

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Purpose of the Study: Erythroderma is a serious condition arising from a variety of dermatological conditions. Skin biopsy is often performed but a significant overlap in features is often seen, regardless of the underlying cause. A retrospective analysis of all cases of erythroderma biopsied in a tertiary dermatology centre over a 2 year period was performed to try and identify single or combined patterns of morphological findings to help distinguish between entities.
Method: Cases were identified via clinical information in the laboratory system. In each case, a panel of 16 morphological features was assessed and scored (on a scale 0-2) including tissue reaction pattern, nature and distribution of inflammatory infiltrate and presence or absence of additional specified findings. If possible, a final or favoured histological diagnosis was made. The final clinical diagnosis for each case was identified via clinical records. Further analysis was performed on cases with a final consensus clinical and histological diagnosis (the assumed 'correct' diagnosis).
Summary of Results: In total, 21 cases were assessed. In 13/21 cases, there was a consensus between the clinical and histological diagnosis: 5 cases eczema, 4 cases psoriasis, 2 cases drug reaction, 2 cases pityriasis rubra pilaris (PRP). On analysis of these cases, only one feature (alternating parakeratin and orthokeratin in both planes) was felt to be specific for a particular diagnosis (PRP). Combinations and severity of the features was however variable across the conditions.
Conclusions: Biopsy in cases of erythroderma can show overlap in features. Combinations of findings may be most helpful towards limiting the otherwise broad differential. In view of this, clinical and pathological correlation is of utmost importance in providing a final consensus diagnosis.

P125

Skin Punch Biopsies "Suspicious" for Squamous Cell Carcinoma: How Suspicious Should we be?

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When there is uncertainty regarding the clinical diagnosis of Squamous Cell Carcinoma (SCC), a non-excisional punch biopsy is commonly performed. In our institute, a proportion of such cases are labelled as "suspicious for a SCC" by the reporting pathologist. This study aims to determine what proportion of these lesions are confirmed to be SCCs in subsequent excisional specimen, and compare this to cases where the original biopsy was considered diagnostic of SCC. A search generated all skin punch biopsies coded "Squamous cell carcinoma"/"Atypia, suspicious for malignancy" at the Royal Victoria Infirmary Newcastle upon Tyne, between 1st January 2015 and 1st January 2016. The reports for any subsequent excisional specimen were also retrieved. 38 non-excisional punch biopsies were labelled "suspicious" for SCC; 26 of these patients underwent subsequent excision of the lesion at our centre. 274 non-excisional skin punch biopsies were diagnosed as SCC; 241 of these patients underwent subsequent excision at our centre. Of those labelled as "suspicious for malignancy", 15/26 (57.8%) of the excision specimens confirmed the presence of a SCC; this compares with 200/241 (83.0%) which were initially deemed diagnostic of a SCC on punch biopsy. 2/26 (7.7%) of cases excised which were called suspicious on punch biopsy were given an alternative diagnosis in the excisional specimen, with one case remaining as suspicious; this compares with a rate of 4/241 (1.7%) when punch biopsy was deemed diagnostic of a SCC. The higher rate of alternative diagnoses found in excisional specimens which were initially labelled as suspicious of a SCC, compared to those cases initially deemed diagnostic, suggests that an appropriate distinction is being made between these two categories. The level of certainty regarding the presence of an invasive component in a punch biopsy specimen should be heeded by the requesting clinician, and prompt careful clinicopathological correlation prior to treatment.

P126

A Novel Method for Screening for Multiple Targets in Tumours Using High Resolution Melting

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Purpose of the Study: We developed the Quick Multiplex Consensus PCR (QMC-PCR) followed by High Resolution Melt Analysis (HRMA) method for mutation screening in samples with low quality template. The method has two-stages: a pre-diagnostic multiplex reaction (PDM) and a singleplex specific diagnostic (SSD) reaction. We sought to modify the method to allow testing of multiple targets.
Materials and Methods: The PDM reaction was retained without change. For the second stage, in-silico design and in-vitro testing allowed us to identify targets with well-separated melting temperatures. About 15 colorectal cancer (CRC) samples which have been enriched in 11 exons of target genes by the PDM reaction were tested. For multiplexed specific diagnosis (MSD) reactions, KRAS exon 3, PIK3CA exon 20 and PTEN exon 3 were chosen. The degree of agreement between the novel MSD and the standard SSD was tested by the crude percentage concordance.
Results: Multiplex PCR produced 3 distinct peaks for KRAS exon 3, PIK3CA exon 20 and PTEN exon 3. Normalization of each peak was performed with the Call-IT software by placing the cursors astride individual raw melt curves. The results of multiplexing the second stage of the QMC-PCR agree well with those of the SSD reactions with 93.3% concordance.
Conclusion: Multiplexing the second stage of the QMC-PCR protocol is possible and can significantly reduce workload and costs of mutation screening.

P127

A Simple Strategy for Delineating Allelic Imbalance in Tumours Without Requiring Normal Tissue

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Purpose of the Study: Tumours frequently show allelic imbalance (AI) at gene loci. If a locus shows heterozygosity for germline single nucleotide polymorphisms (SNPs), then AI will result in a change in the ratio of the SNPs, a move towards homozygosity and creation of a minor allele. The latter feature allows AI to be tested by comparing tumour and matched normal tissue using High Resolution Melting Analysis (HRMA). When normal tissue was not available, it may be possible to use forced change of minor allele frequency (MAF) to detect AI.
Method: DNA from tumour and matched normal tissue (n= 20 each) containing a germline SNP (at SMAD4 SNP rs12455792) was amplified using conventional PCR. The MAF was altered in the samples using Co-Amplification at Lower Denaturation temperature (COLD) PCR which would result in enrichment of the minor allele. The products were subjected to Sanger sequencing and to HRMA on the LightScanner-96 platform.
Result: Comparison of conventional PCR and COLD-PCR products showed alterations of the MAF in tumour samples. This was manifest in the HRMA by an increase in the height of the peak following COLD PCR. Sanger sequencing confirmed this and an increase in the height of the peak of the C allele following COLD PCR was seen. The matched normal tissue showed no change in either HRMA or sequencing following COLD-PCR.
Conclusion: In the absence of available normal tissue, AI can be detected in tumours by comparing PCR products obtained by conventional and COLD PCR using HRMA to detect shifts in MAF.

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Aberrant High Resolution Melting Pattern: Germline Variant or Somatic Mutation?

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Purpose of the Study: High Resolution Melt Analysis (HRMA) is a simple mutation screening technique. However, HRMA cannot distinguish single nucleotide polymorphisms (SNPs) from somatic mutations. We hypothesised that, due to stromal contamination, somatic mutations would usually represent minor alleles. These could be enriched by COLD-PCR and detected by a change in melting pattern whilst this would not happen with SNPs.
Method: Multiple tumour samples with known aberrant HRM pattern due to SNPs (n=10) or somatic mutations (n=10) in the POL E gene were subjected to both the Quick Multiplex Consensus PCR and the COLD PCR. The products were melted on the LightScanner96 and analysed. Sanger sequencing was utilised to validate the HRMA findings.
Results: In the samples with somatic mutation, COLD PCR caused mutation enrichment leading to a difference in melting pattern between the product of the QMC-PCR and the COLD PCR. The enrichment of the minor allele was confirmed by sequencing. In contrast, sample with germline SNP showed no difference in the melting pattern between the products of QMC-PCR and COLD PCR. To further verify this, SNP containing DNA form matched normal tissue was tested. Analysis showed that there was no difference in the melting patterns of either the normal or tumour DNA.
Conclusion: Germline SNPs can be distinguished from somatic mutation in a tumour DNA sample by simply comparing the melting pattern of the product amplified by the standard and COLD PCR. Mutation screening in tumours can therefore be performed in the absence of matched normal tissue.

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Morphology Assessment and Suitability for Diagnosis of Formalin-fixed Versus PAXgene Tissue-fixed and Paraffin Embedded Tumour Tissue

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Purpose of the Study: To investigate whether fixation using the PAXgene Tissue system provides cellular and tissue morphology comparable to that seen in formalin-fixed paraffin-embedded (FFPE) tissue and suitable for diagnostic purposes.
Methods: 36 paired tumour or normal tissue samples were obtained from 18 fresh surgical resection specimens and fixed using either 10% neutral buffered formalin or PAXgene Tissue system. All tissue samples underwent automated overnight processing and paraffin wax embedding. H&E sections were produced and independently assessed in a blinded manner by two pathologists. Nuclear, cytoplasmic and other tissue components were scored to give a maximum possible score of 12 for each case.
Results: The pathologists showed 94% concordance (17/18 cases) in predicting which was the PAXgene Tissue-fixed and paraffin-embedded (PFPE) tissue sample of each pair. These could be easily identified due to increased intensity of eosin staining and swelling and central clearing of erythrocytes, both recognised artefacts in other studies using PAXgene Tissue system. PFPE samples also showed increased fragility, particularly in necrotic areas where tearing of sections was more commonly seen. Average scores for PFPE (10.7 for pathologist 1 and 9.6 for pathologist 2) were slightly lower than those for FFPE tissue (11.8 for both pathologists). Lymph nodes showed inferior preservation in PAXgene Tissue, with cell shrinkage and tissue disaggregation as well as slightly less crisp nuclear features. Overall both pathologists assessed all FFPE as suitable for diagnosis and 89% of PFPE sections (16/18) as suitable for diagnosis.
Conclusions: PAXgene Tissue fixed samples are easily recognisable in H&E sections and overall show comparable morphology to formalin-fixed samples, which is generally suitable for diagnostic purposes. Our early results suggest that lymphoid tissue show slightly inferior preservation which may affect the diagnostic process.

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A Comparison of Somatic Mutation Calling in Fixed Tumour Tissue Between the Affymetrix OncoScan Array and a PCR-based Next-generation Sequencing Approach

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Purpose of the Study: The importance of accurate and affordable somatic mutation (SM) calling in histo-pathological samples is increasingly important as we move into the era of personalised medicine. We compared SM calls made in clinical material using the Affymetrix OncoScan Array platform with calls made using a custom designed PCR panel followed by next-generation sequencing (NGS), in order to prove concordance of sensitivity and specificity over a range of variant allele frequencies (VAF).
Methods: FFPE tumour samples (n=387) were analysed on the OncoScan platform using recommended protocols, which along with genome-wide copy number, also tests for the presence of 74 SMs. The same samples were subsequently sequenced from PCR products covering all SMs on the array. Each SM was covered by two overlapping PCR primer pairs to provide redundancy and internal replication. PCR products were pooled and sequenced on an Illumina MiSeq.
Summary of Results: From the 387 FFPE samples, we tested 302 Oncoscan positive mutation calls spanning 35 different SMs and 1175 negative calls spanning 45 different SMs. The sensitivity and specificity were both >95% compared to the NGS results. We were unable to compare some of the rarer SMs. Most discrepancies were limited to a small number of specific SMs, or samples with failing QC values. All high quality SMs that were consistent between the platforms were called in both PCR reactions.
Conclusions: Good concordance was demonstrated between the OncoScan platform and the NGS approach. While some SMs were discordant, many of these were large insertions/deletions which are not easily captured by PCR/NGS. Sample quality was the next biggest cause of discrepancy, confirming that the quality of fixation is very important. Overall, there was still good concordance with borderline QC samples, and for mutations with VAF above 10%. Both the OncoScan platform and the NGS approach were able to provide trustworthy SM calls for the majority of samples.