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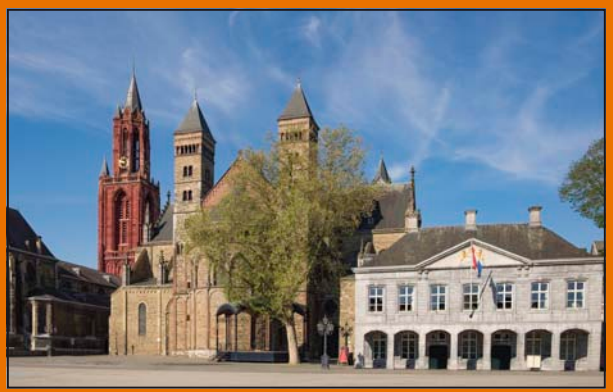

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P1

Megakaryocytes in Axillary Lymph Nodes of Breast Carcinoma Patient Treated with Neoadjuvant Chemotherapy: A Potential Diagnostic Pitfall

Ⓟ NM Badr[1]; C Roberts[2]; AM Shaaban[3]

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Purpose of the study: The presence of megakaryocytes in the axillary lymph node of breast carcinoma patients is extremely rare but when encountered can represent a diagnostic challenge. We aim to highlight this incidental finding as a diagnostic pitfall which can be mistaken for metastatic carcinoma (particularly of the metaplastic type).
Methods: We report a case of a 68 years old Caucasian female smoker with family history of cancer. Core biopsy revealed grade II oestrogen receptor negative, Her2 positive invasive ductal carcinoma. She was offered neoadjuvant chemotherapy (NACT) with Herceptin and subsequently underwent breast-conserving surgery.
Summary of results: Microscopic examination of post treatment breast surgical specimen showed partial pathological response with wide areas of tumour regression. The sentinel lymph node showed frequent large single and multinucleate giant cells with hyperchromatic nuclei located predominantly within the subcapsular and medullary sinuses. The morphological differentials of metastatic carcinoma, sinus histiocytosis and extra medullary haematopoiesis were considered. A panel of immunohistochemistry showed these large cells to be negative for epithelial markers and CD68. They were strongly positive for CD42b (megakaryocyte marker). Smaller myeloperoxidase and factor VIII positive cells were identified. The findings confirmed extramedullary haematopoiesis.
Discussion and Conclusions: Sentinel nodes are often well scrutinised by pathologists for evidence of metastatic carcinoma as an important prognostic parameter both in the standard and neoadjuvant setting. Nodal megakaryocytes have been described in response to NACT particularly in association with Herceptin treatment. Pathologists' awareness of this finding in the neoadjuvant setting is crucial to avoid a mistaken diagnosis of malignancy. Approach of relevant immunohistochemical panel together with careful attention to morphology should help establish the correct diagnosis.

P2

Atypical Vascular Lesions and Angiosarcomas of Breast: Experience of a Large Tertiary Centre

Ⓟ R Merard; N Deshmukh; A Shaaban

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Radiation induced vascular lesions are rare but diagnostically challenging lesions. We present an audit of breast angiosarcomas and atypical vascular lesions (AVL) reported in a large breast oncoplastic and sarcoma centre to assess the diagnostic utility of c-myc immunohistochemistry in those lesions. All cases of angiosarcoma of the breast during the period from 2013 to 2017 were included. Data was collected from the histopathology database. Histological reports and selected slides were reviewed by breast and soft tissue pathologists. Comprehensive clinical and pathological data were collated and statistically analysed. Of the total 33 cases, one was a primary angiosarcoma, 4 were AVLs and 28 cases were radiation-induced angiosarcomas (RIAS). In the RIAS, the primary surgery was wide local excision in 25 cases and mastectomy in 3 cases. The interval between the diagnosis of primary breast cancer and angiosarcoma ranged from 4 years to 30 years with a median of 8 years. 45% of the cases presented with a mass lesion, while 33% presented as a cutaneous lesion. The tumour was confined to skin in 11 cases and localised to breast parenchyma in 6 cases. 7 cases showed both skin and parenchymal involvement. 52% of cases showed a high-grade spindle cell morphology, while the rest exhibited an epithelioid morphology. All, except one case of RIAS, showed strong positive nuclear staining for c-myc. c-Myc was negative or weak in all AVLs and in the primary angiosarcoma case. 8 patients with RIAS and one case of primary angiosarcoma died within 2 years of diagnosis. c-Myc immunohistochemistry is a reliable and useful diagnostic tool in the assessment of radiation-induced vascular lesions. A strong expression strongly favours RIAS. It can also be used to assess the margin status and tumour extent of those frequently infiltrative tumours. Larger studies are required to further assess its diagnostic role in ruling out primary angiosarcoma/AVL and its prognostic significance in RIAS.

P3

Anaplastic Large Cell Lymphoma Presenting as Extra Mammary Disease in Two Patients with Long-Term Breast Implants

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[1]University of Birmingham, Birmingham, UK; [2]Queen Elizabeth Hospital Birmingham, Birmingham, UK

Purpose of study: The association between breast implants and anaplastic large cell lymphoma (ALCL) has been recognised. All previously reported cases presented with implant thickening and mass/effusion. We report two cases of high grade ALCL which unusually presented as mediastinal, cervical and axillary lymphadenopathy in two females with bilateral breast implants.
Case summaries: The first patient is a 35-year-old female with a 10-year history of bilateral implants. She presented with right cervical, superior mediastinal, paratracheal and inframammary chain lymphadenopathy. Nodal excision confirmed a CD30 positive, ALK negative ALCL. There was positivity for both B cell markers PAX5, CD79a as well as T cell markers CD4, CD3, CD2 and CD5. IgH and TCR gene rearrangement studies demonstrated B and T cell clonality. She underwent CHOP chemotherapy regimen followed by Brentuximab and achieved complete metabolic remission. Her breast implants showed no evidence of ALCL histologically. The second patient is a 50-year-old who had bilateral breast augmentation with implants 20 years ago. There was no imaging evidence of capsular rupture. A group of right axillary lymph nodes were abnormal. The diagnosis of ALK negative ALCL was made upon excision of a right axillary lymph node. The large atypical cells were positive for CD30, MUM1, EMA, CD4 and BCL2. Histologically, the breast capsules showed no evidence of ALCL. The patient received 6 cycles of CHOP chemotherapy and there is no evidence of disease progression after 11 months of completion.
Conclusion: Our cases highlight the potential atypical presentation of ALCL as extra-mammary lymphoma. Case 1 is noteworthy as despite presenting with high stage disease, the patient achieved complete metabolic remission. The patient was treated presumptively as breast implant associated ALCL and did not receive consolidation with transplant, which would be the standard of care for non-breast implant associated ALK negative ALCL.

P4

Gastric Cancer Synchronous or Metachronous with Breast Cancer: Primary Tumour or Metastatic Disease?

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The incidence of breast cancer (BC) is high and over the years survival has improved. Consequently, BC survivors are at risk of getting a second primary cancer. However, distinction from metastatic BC is required since treatment is widely divergent. Among these secondary primary cancers the distinction between primary gastric cancer (pGC) and gastric metastasis of BC (gmBC) can be challenging. It is known that gmBC can be mimicker of pGC, clinically, at endoscopy and histologically. We present an overview of BC patients with gastric cancer to gain more insight in the clinicopathological presentation.
Methods: Retrospective review of single centre based results of patients with gastric cancer and a history of BC. Patients were identified from the local pathology database (period 1988-2018). We determined characteristics of patients, BC and treatment. Secondly, we identified the clinical presentation, characteristics and treatment of gastric cancer.
Results: 38 patients were included. The interval between diagnosis of BC and presentation of gastric symptoms ranged from synchronous to 28 years later. There were no significant differences in the clinical presentation of gmBC and pGC: the interval in months was 63 ± 64 (gmBC) vs 97 ± 81 (pGC) (p=0.833). 12 patients (32%) had been diagnosed with gmBC, whereof in 2 cases a diagnosis of gmBC was made only after stomach resection. In at least 46% the possibility of gmBC was not considered. Histologic BC type differed significantly between patients with gmBC and GC: invasive lobular carcinoma 63% (gmBC) vs invasive ductal carcinoma 67% (p=0.035).
Conclusion: Our data supports the existing evidence of gmBC: invasive lobular carcinoma is overrepresented and the clinical and timing of presentation cannot be distinguished from a pGC. In addition we show that 1 in 3 gastric cancers in BC patients is gmBC. Therefore, in case of a gastric cancer in a patient with a history of BC, gmBC should always be considered

P5

How Representative are Tissues Donated to Specialist Breast Cancer Biobanks? A Retrospective Analysis of the Breast Cancer Now Tissue Bank

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Background: Specialist tissue banks are a valuable resource for researchers, facilitating access to both tissue and longitudinal clinical follow up data. The Breast Cancer Now Tissue Bank (BCNTB) provides access to both fresh frozen and formalin fixed samples donated by breast cancer patients. An issue facing biobanks is how truly representative these samples actually are; according to the NCIN the average size of excised breast tumours has significantly decreased in the last 20 years. It has been hypothesised that sampling methods, and the need to retain sufficient tissue for diagnostic purposes, may result in a bias towards larger tumours being banked. This study aimed to compare the sizes of tumours banked by BCNTB with published national data, to see if samples were truly representative of breast cancers nationwide.
Methods: A retrospective analysis was performed on 2415 patients who consented to donation to the Leeds and Barts BCNTB from 2010-2018. Data was retrieved from electronic patient records on invasive tumour size. Patients who had undergone neoadjuvant therapy were excluded. Invasive tumour sizes for screened and symptomatic cases were then compared with national NCIN data from 2006 and 2011 in predefined categories of <15mm, 16-20mm, 21-35mm, 36-50mm, and >50mm.
Results: The data collected from Leeds and Barts were comparable in both screened and symptomatic cases. As expected, the screened cohort had a greater number of cases <15mm, and symptomatic patients had an increased proportion of tumours >20mm. Overall, 58.3% of screen detected breast cancers were <15mm, compared with 52.8% nationally. For symptomatic breast cancers, 36.1% were <15mm, compared with 22% nationally.
Conclusion: We found no evidence of bias towards the banking of larger tumours using the pre-established size groups. However, the most recent national data was from 2011, hence our data may need to be compared with more recent figures. With smaller tumours being detected through screening (e.g. <10mm), future work should examine strategies to usefully bank these cases.

P6

A Rare Breast Entity: Solitary Neurofibroma of the Breast

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We present a case of a benign nerve sheath tumour of the breast diagnosed on needle core biopsy in a 47 year old female patient who was symptomatic with a solitary, palpable left breast mass. Histology revealed a bland spindle cell lesion with a prominent myxoid background and a meshwork of collagen fibres. Cytologically the spindle cells had ill defined pink cytoplasm and wavy nuclei with tapered ends. Throughout the lesion scattered mast cells were noted. There was no necrosis present. Cytolgical atypia and mitotic figures were not a feature. Immunohistochemistry was performed and the lesion showed strong diffuse positivity for S100. The lesion showed negative immunoreactivity for beta-catenin, smooth muscle actin, Desmin and the pan-cytokeratin MNF-116. This immunohistochemistry panel was supportive of a diagnosis of Neurofibroma. Neurofibromas are relatively frequent within the soft issues but this is a rare diagnosis within the breast, particularly if there is no association with Neurofibromatosis type 1. Neurofibromas commonly present as a painless masses and demonstrate benign behaviour therefore surgical excision is curative. We present this case report of a solitary breast Neurofibroma and discuss this finding within the context of Neurofibromatosis and the relevance of Neurofibromatosis on breast pathology. We also discuss the differential diagnosis of spindle cell lesions within the breast and how immunohistochemistry can assist in the diagnostic algorithm.

P7

Phyllodes Tumour of the Breast: A 10-Year Single-Centre Retrospective Analysis and Impact of Surgical Margins

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Objective: There is no consensus as to what constitutes a surgically excised clear margin in Phyllodes tumour (PT), which encompasses a wide range from 1mm - 10mm. We aimed to examine the histopathological data and clinical follow-up of all patients diagnosed with PT on excision following core biopsy at our Breast Unit in Charing Cross Hospital London from January 2007 to December 2017. We analysed (A) cases with complete excision >/= 1mm margin; (B) cases with <1mm or involved margins and whether further re-excision or 'watchful waiting' policy was adopted and (C) the recurrence rate.
Method: A total of 73 patients were retrieved from the electronic records (Co-Path LIMS and Cerner); 72 females and 1 male, age 18 years to 81 years (median=43) with follow-up of 6 months to 10 years. Of these, 56 (76.7%) had benign PT, borderline 8 (11.0%) and malignant 9 (12.3%). 8 patients were lost to follow up. The study was conducted on 65 final patients (benign=52; borderline=7; malignant=6).
Results: Wide local excision was the main treatment in benign 52 category; 27 had complete excision (>/=1mm); 25 incomplete of which 16 underwent revision surgery with clear margins, 8 had 'watch-and-wait' policy and 1 patient declined treatment. In borderline cases 2 had complete, 5 with incomplete removal and clear margins re-excision. The narrowest margin recorded was 1mm. For malignant, mastectomy and sentinel node biopsy in 2 cases; 4 cases had wide local excision and clear margins. All patients followed up for five years. Malignant cases followed sarcoma protocol at a different institution. Three recurrences seen at 4months-1 year, sizes 30mm, 40mm and 70mm; 2 in benign and 1 in borderline; 2 originally completely excised. None recorded in malignant PT.
Conclusion: We conclude that in selected cases, wide local excision with narrow margins and 'watch-and-wait' policy even in positive/involved margins for benign phyllodes tumour is an acceptable treatment option.

P8

Non-Receptor Tyrosine Kinase *SRC* (*c-SRC*) is an Independent Predictor of Local Recurrence in Breast Ductal Carcinoma In Situ (*DCIS*)

Ⓟ IM Miligy; M Toss; A Al-Kawaz; CC Nolan; M Diez-Rodriguez; IO Ellis; AR Green; EA Rakha

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Purpose of the study: Current clinical and pathological parameters are important predictors of recurrence in breast ductal carcinoma in situ (DCIS), but they are insufficient to reflect its molecular heterogeneity. The non-receptor protein tyrosine kinase SRC plays a crucial role in the signal transduction pathways involved in cell division, motility, adhesion, and survival in both normal and cancer cells. The prognostic significance of SRC was investigated in some human malignancies including invasive breast cancer, however its role is not confirmed in the pre-invasive stage. This study aims to assess the prognostic role of SRC in DCIS.
Methods: Tissue microarrays from 776 pure DCIS and 239 mixed DCIS and invasive tumours were constructed. All patients were treated in a single institution between 1990 and 2012. Patients' clinical information, management and follow-up data were retrospectively collected. The expression of SRC was assessed immunohistochemically and correlated with the clinicopathological parameters.
Results: Pure DCIS lesions showed lower expression of SRC compared with DCIS lesions associated with IBC (p=3x10-4). In pure DCIS, high SRC expression was associated with features of aggressiveness including high nuclear grade (p=1.7x10-8), multifocal tumours (p=0.001), presence of comedo necrosis (p=0.006) and hormone receptor positive (ER+/PR+)/HER2+ (triple positive) DCIS (p=0.004). Univariate outcome analysis showed positive association with the development of local invasive recurrence (p=0.007) and was a predictor of local recurrence (p=0.041, HR=1.696 and 95%CI: 1.004-2.864), independent of size and nuclear grade.
Conclusions: SRC expression predicts local recurrence in DCIS patients and is potentially useful in prognostic stratification of DCIS patients for management decisions.

P9

Glutaminase Expression is Potentially Driven by Alternative Mechanisms in the Molecular Subtypes of Breast Cancer

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Purpose of the Study: Altered cell metabolism is one of the hallmarks of cancer where cancer cells regulate their own metabolism to provide energy required for growth. Many cancer cells are highly reliant on the amino acid glutamine for sustained cellular proliferation and survival and some solid tumours become addicted to this amino acid. Breast cancer (BC) is a heterogeneous disease and glutamine dependent mechanisms can vary substantially among the different BC subtypes. Glutaminase (GLS) is a key enzyme in glutamine metabolism and is highly upregulated in triple negative and HER2+ BC. We hypothesise that mechanisms of GLS upregulation vary in the different molecular subtypes of BC and may further guide targeted therapy.
Methods: GLS1 expression was assessed in large, well-characterised BC cohorts at the DNA, mRNA and protein level (METABRIC, n=1980; Nottingham Series, n=1192) and correlated with clinicopathological parameters, c-Myc and other regulatory proteins with consideration of molecular subtypes.
Summary of Results: High GLS1 mRNA and protein were expressed in 49.7% and 41.6% tumours, respectively. Gain of GLS1 CN, high GLS1 mRNA and high GLS1 protein expression were associated with negative oestrogen and progesterone receptor expression (p<0.001). High expression of GLS1 protein was positively associated with proliferative proteins and c-Myc expression (p<0.001). GLS1 protein was positively associated with high PIK3Ca expression in ER+/HER2- high proliferation subtype (p=0.008), high mTORC1 in HER2+ tumours (p=0.003) and high p53 expression in TN tumours (p=0.008).
Conclusions: GLS1 expression is potentially driven by alternative mechanisms in molecular subtypes of BC. More specifically, PIK3Ca is a potential regulator of glutaminolysis in the aggressive subclass of luminal BC and mTORC1 in HER2+ BC. We propose using a variety of in vitro functional assays to validate our observational findings and assess their value as therapeutic targets in BC.

P10

X-Ray Repair Cross-Complementing Gene 1 (XRCC1) is a Predictive Marker of Poor Prognosis in Ductal Carcinoma In Situ (DCIS)

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Introduction: The role of DNA repair in cancer has been extensively studied. Impaired DNA repair could contribute to poor prognosis. XRCC1 is a key player in Base excision repair (BER) and single-strand breaks repair (SSBR). XRCC1 interacts with PARP1 and coordinates BER. Inhibition of PARP1 in XRCC1 deficient tumours can be an attractive synthetic lethality approach.XRCC1 deficiency delays SSB re-joining leading onto (SSBs) and if unrepaired, eventually to double-strand breakers (DSBs). XRCC1 deficiency can also hyper activate PARP1. Our hypothesis that XRCC1 downregulation is an early event in breast cancer pathogenesis.
Methods: A cohort of 779 cases of pure DCIS and 239 cases of mixed DCIS/IBC were arrayed in tissue microarrays. XRCC1 protein expression was assessed using immunohistochemistry. Correlated with clinicopathological parameters and patient outcome.
Results: XRCC1 was highly significant (P<0.001) with low expression, within pure DCIS (79.9% of cases), in DICS associated with IBC (60.7%). In the pure DCIS cohort, low expression of XRCC1 correlated with age>45 years (P=0.048), ER status (P=0.015). In the mixed cases, lower expression of XRCC1 is seen in invasive component than in DCIS component (P<0.008), however, no significance seen with patient's outcome. XRCC1 deficiency pre-invasive DCIS are aggressive and link to increased risk of local recurrence. In addition, XRCC1 deficiency with high PARP1 levels manifest aggressive feature and poor survival.
Conclusion: These results suggest that loss of XRCC1 expression is associated with aggressive DCIS, also we conclude that PARP1 targeting is an attractive synthetic lethality and chemoprevention strategy in XRCC1 deficient invasive cancer or DCIS.

P11

Immune Checkpoint Proteins Evaluation in Triple Negative Breast Cancer

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Introduction: Patients with Triple negative breast cancer (TNBC) are said to have poor prognosis than those of other subtypes. However, since they do not benefit from hormone therapy there is the need to identify other markers for use in targeted therapy. Overexpression of the immune checkpoint proteins (ICP); Programmed Death 1 (PD1) and its ligand, Programmed Death Ligand (PDL1); have been established in other tumours with objective responses to anti-PD1/anti-PDL1 therapy being reported in clinical trials.
Aim: This study investigates the expression of PD1 and PD1L1 in TNBC and its association with other inflammatory markers and clinicopathological parameters. Materials and Methods: Immunohistochemistry on 75 full face sections of TNBC was used in evaluating the expression of PD1 and PDL1 at both tumour and tumour infiltrating inflammatory cells.
Results: Membranous and/or cytoplasmic PDL1 expression on tumour and inflammatory cells were significantly associated with CD68, FOXP3 and CD8+ T cells ($p < 0.05$). For membranous PD1 expression on inflammatory cells, a significant association was found with tumour grade ($p = 0.02$). A significant correlation was observed between ICP expression on tumour and inflammatory cells ($p = 0.002$).
Conclusions: The expression of PDL1 in triple negative breast cancer can be explained by PD1 expression on inflammatory cells. Also, PDL1 could be further investigated as novel immunotherapy targets in treatment of TNBC.

P12

Legumain (LGMN) is an Independent Poor Prognostic Factor in Breast Ductal Carcinoma In Situ (DCIS)

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Background: Legumain (LGMN) is a proteolytic enzyme and plays role in regulation of cell proliferation. LGMN is differentially expressed at mRNA level between breast ductal carcinoma in situ (DCIS) and invasive breast carcinoma (IBC). Here, we aimed to characterise LGMN protein expression in DCIS and evaluate its prognostic significance.
Methods: Tissue microarray (TMA) was constructed from a large cohort of DCIS that have available paraffin blocks with representative tumour tissue (n=776 for pure DCIS and n=239 for DCIS associated with IBC (DCIS/IBC)). TMA sections were stained for LGMN immunohistochemically and scored following robust validation of staining specificity.
Results: High LGMN expression was observed in 22.6% of pure DCIS. High expression was associated with features of poor prognosis including higher nuclear grade, comedo necrosis, HER2 positivity and hormone receptor negativity. High LGMN expression was associated with shorter recurrence free interval (RFI) (p=0.0003). In multivariate survival analysis for patients treated with breast conserving surgery; LGMN was an independent predictor of shorter RFI (p=0.04). DCIS associated with IBC showed higher LGMN expression than pure DCIS (p=0.001). In DCIS/IBC cohort; LGMN expression was higher in invasive component than DCIS component (p=0.02).
Conclusion: LGMN is associated with aggressive behaviour and poor outcome in DCIS through its proteolytic activity and also could be a potential marker to predict co-existing invasion in DCIS.

P13

Breast Cancer Ex Vivo Anthracycline Sensitivity Test (BREAST) Using Organotypic Tissue Slices From Core Needle Biopsies

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Purpose of the study: Optimal patient stratification is of utmost importance in the era of personalized medicine. Prediction of individual treatment responses by *ex vivo* assays require model systems derived from viable tumour samples. By using organotypic slices, taking tumour heterogeneity and tumour-stromal interactions into account, our model closely resembles *in vivo* tumour characteristics and microenvironment. In the pilot 'Breast Cancer Ex vivo Anthracycline Sensitivity Test (BREAST)' study, the predictive value of this test for *in vivo* response is determined.
Methods: Breast cancer (BrC) patients treated with anthracycline-based neo-adjuvant chemotherapy (NAC) are included in this study (required n=20). The primary endpoint is concordance between the *ex vivo* sensitivity test and *in vivo* response to NAC. Pre-treatment core needle biopsies are obtained from the primary BrC. 300µm organotypic tissue slices are prepared using the Leica VT1200 S vibrating blade microtome (Vibratome). Tissue slices are cultured *ex vivo* in customized culture medium under constant rotation with or without addition of chemotherapeutics for up to 1 week. Drug response is evaluated by scoring morphology (HE-staining), proliferation (EdU-incorporation) and apoptosis (TUNEL staining).
Summary of results: The drug sensitivity assay was developed for anthracycline-based treatment on a set of 23 primary BrC. Within one week, differences in intrinsic sensitivity to *ex vivo* treatment were observed. As proof of principle, we identified one clinically proven non-responsive tumour after NAC to be highly resistant in our *ex vivo* assay. To enhance diagnostic potential, we have adapted this technique for core needle biopsies. Currently, 6 evaluable patients have been included in the BREAST study.
Conclusions: Drug sensitivity assays on organotypic slices from core needle biopsies are feasible. Whether the test is predictive for *in vivo* response to anthracycline-based NAC is currently under investigation.

P14

Inter- and Intra-Laboratory Variation in the Histopathological Grading of Invasive Breast Cancer in a Nationwide Cohort in the Netherlands

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Purpose of the study: Histologic grading of invasive breast carcinoma (IBC) is used in all stages of patient management and its clinical contribution has become increasingly important. Yet, the level of inter- and intra-observer agreement does not reach high enough clinical standards. To improve standardization, we aimed to gain insight into laboratory-specific variation of histologic grading.
Methods: All synoptic pathology reports of IBC resection specimens between 2013-2016 were retrieved from PALGA, the nationwide Dutch Pathology Registry. Grade was determined according to the modified Bloom and Richardson guideline. Absolute differences in proportions of grade I-III and the three components of grading between laboratories were compared to the national distributions. Multivariable logistic regression provided laboratory-specific odds ratios (ORs) and 95% confidence intervals (CI) for grade I versus II-III and grade I-II versus III compared to the reference laboratory.
Summary of results: In total 35,549 IBC cases from 39 laboratories were included, of which 28.0% were reported as grade I (range 16.4-43.4%), 47.6% as grade II (range 38.4-57.1%), and 24.4% as grade III (range 15.8-33.8%). After correction for case mix, 23 laboratories (59.0%) had at least one significantly higher or lower OR than the reference laboratory (grade I versus II-III and/or I-II versus III). Seven laboratories (18.0%) had significantly deviant ORs on both analyses. Most variation between laboratories was observed for nuclear polymorphism, followed by mitotic count and tubular formation. Significant grading differences were also observed between pathologists within 62.5% of participating laboratories.
Conclusions: We observed substantial inter- and intra-laboratory variation in the histologic grading of IBC. Interventions to improve histologic grading nationwide should especially aim to standardize nuclear polymorphism, since most variation was observed in this category.

P15

Underestimation of Invasive Breast Cancer in 2,892 Biopsies with Ductal Carcinoma In Situ: A Prediction Model and Characteristics of 589 Invasive Cancers

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Background: Patients with a biopsy diagnosis of ductal carcinoma in situ (DCIS) might be diagnosed with invasive breast cancer at excision, a phenomenon known as underestimation. This study aimed to expand the knowledge on underestimation and to predict the underestimation of invasive cancer.
Methods: Population-based data were retrieved from the Dutch Pathology Registry and the Netherlands Cancer Registry for DCIS between 2011 and June 2012.
Results: Of 2,892 DCIS biopsies, 21% were underestimated invasive breast cancers. In multivariable analysis, risk factors were high grade DCIS (OR 1.43, 95%CI 1.05-1.95), a palpable tumour (OR 2.22, 95%CI 1.76-2.81), a BI-RADS score 5 (OR 2.36, 95%CI 1.80-3.09), and a suspected invasive component at biopsy (OR 3.84, 95% CI 2.69-5.46). The predicted risk for underestimation ranged from 9.5% to 80.2%, with a median of 14.7%. Of the 596 invasive cancers, 39% had unfavourable features.
Conclusions: The risk for an underestimated diagnosis of invasive breast cancer after a diagnosis of DCIS at biopsy is considerable. With our prediction model, the individual risk of underestimation can be calculated based on routinely available pre-operatively known risk factors. The model can be used to reduce overtreatment: patients with low risk can be selected for active surveillance trials, while only patients with a high risk for underestimation can be selected for sentinel lymph node biopsy.

P16

The Utility of Transbronchial Lung Cryobiopsy in Interstitial Lung Disease[ILD]: Experience of an Irish Tertiary Referral Centre

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Introduction: Multidisciplinary consensus diagnosis (MDT CD) is recommended for ILD with correlation of clinical, imaging and surgical lung biopsy (SLB) findings. Although reliable, SLB is invasive with associated risks and costs. Transbronchial lung biopsy (TLB) is limited due to small size and artefacts. Transbronchial lung cryobiopsy (TLC) has been advanced as a less invasive option yielding better quantity and quality alveolated lung samples than standard TLB.
Objective: To assess the usefulness of TLC in achieving MDT CD during ILD investigation.
Methods: Retrospective review of all TLCs performed in a tertiary insitute from 2014-2016. Tissue size, adequacy, complications and diagnostic yield were analysed.
Results: 75 patients underwent 90 TLCs yielding 246 cryobiopsies (2.73 biopsies/case), mainly from RLL (60.6%). Mean biopsy diameter: 6.1mm (range 3-22mm). 96.7% biopsies contained alveolated lung. MDT CD was achieved in the majority of cases (84%). In a minority of cases (13.3%), the histological findings resulted in consideration of an alterative diagnosis to the initial clinico-radiological impression. Pneumothorax and moderate bleeding occurred in 15 cases (16.7%) each. 7 patients required hospital admission, mean stay 1.3 days.
Conclusion: Early data on TLCs appears promising, however, further studies are needed and SLB currently remains the recommended procedure in ILD investigation.

P17

Sample Adequacy of Endobronchial Ultrasound-Guided Fine Needle Aspiration (EBUS) Investigations of Mediastinal and Hilar Lymphadenopathy: Review of 500 Benign Cases Over an 8-Year Period

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The Endobronchial Ultrasound-Guided Fine Needle Aspiration (EBUS) sampling technique of mediastinal and hilar lymph nodes has a sensitivity of 100% and a specificity of 94% in lymphadenopathy diagnosis. This method's diagnostic accuracy for sarcoidosis compares favourably to other more invasive techniques such as a transbronchial lung biopsy, and mediastinoscopy, making EBUS the gold standard for sarcoidosis diagnosis. Our institution has performed EBUS procedures since 2010 and has implemented a Rapid On-Site Evaluation (ROSE) procedure by a cytopathologist with the aim of reducing non-diagnostic sampling. A retrospective audit of benign EBUS performed between 2010 and 2017 was performed with analysis including number performed, implementation of ROSE and sample adequacy, assessed by viewing the individual cytopathology report of each procedure. 500 EBUS were performed on 472 patients in the 8-year period for the indication of suspected malignancy and sarcoidosis. Of the cases where ROSE was not performed 15.85% of samples were inadequate while when ROSE was performed 12.56% of samples were inadequate. Lymph node station 7 was the most commonly sampled (65.67%), with an inadequacy rate of 26.5%, followed by 4R (frequency of sampling 28.39%, inadequacy rate 23.4%), and 4L (frequency of sampling 11.01%, inadequacy rate 21.8%). The implementation of ROSE in EBUS has reduced the inadequate sampling of mediastinal and hilar lymph nodes, providing benefits for both the pathology department and the patient in terms of duration of procedure, cost, and efficiency.

P18

Concordance Rate Between Histologic and Cytologic Specimens in Lung Cancer Diagnosis

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Purpose: Limited diagnostic tissue may be available for tumour subtyping and determination of subsequent therapeutic pathways in lung cancer. We compare the accuracy of preoperative cytology and small biopsy histology specimens in the assessment of suspicious lung masses.
Methods: A retrospective database search over a two year period (2016-2017) for all cases of respiratory cytology with concurrent lung biopsy and subsequent lung resection was performed. We investigated concordance rates between preoperative histology/cytology and final surgical histologic subtype. Concordant diagnoses were those where preoperative cytology/histology diagnostic subtype tallied with the final histology subtype. A minor discordance was recorded when there was variance between preoperative histology/cytology tumour subtype and that of the final histologic specimen. A major discordance was recorded when there was a benign/malignant mismatch between the preoperative histology/cytology specimen and the surgical resection histology.
Results: Thirty cases were included for assessment. Thirteen cases (43%) showed concordance between both preoperative histology and cytology and the final surgical histology (ten squamous cell carcinomas, three adenocarcinomas). Seven had concordant diagnosis on lung biopsy alone (23%), with concurrent major discordance between cytology and surgical resection diagnosis, (one large cell carcinoma, five adenocarcinomas, one small cell carcinoma). Four tumours (13%) showed concordant lung biopsy/surgical resection subtyping as adenocarcinoma. In these cases there was concurrent minor discordance on cytology. Two cases (7%) showed concordance with the final surgical histology on cytology alone (one benign hamartoma and one mixed squamous cell/neuroendocrine carcinoma). Four cases (13%) showed discordance between the final surgical histology and both preoperative biopsy and cytology.
Conclusions: Concordance with final surgical resection diagnosis was high (87%).

P19

Nuclear Localisation of Annexin A1 May Promote an Invasive and Metastatic Phenotype in Lung Adenocarcinoma

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Purpose: Annexin A1 (ANXA1) promotes invasion and metastasis in many cancers, acting through various intracellular and extracellular pathways. Previous studies have demonstrated an association between ANXA1 overexpression and disease progression in non-small cell lung cancer, but there is little work on the effect of its localisation within the cell. This study aims to evaluate a role for both overall ANXA1 expression and its subcellular localisation in the progression of lung adenocarcinoma from in situ to invasive and metastatic disease, as well as correlating this with epithelial-to-mesenchymal transition (EMT).
Methods: Surgical resection specimens of lung adenocarcinoma were assigned into three groups: lepidic pattern-only (n=14), invasive tumour with no nodal metastases (n=20) or metastatic carcinoma (n=20). Total ANXA1 expression and intracellular localisation (nuclear, cytoplasmic or membranous) were evaluated by immunohistochemistry in both lepidic and invasive components of each individual tumour, as well as in matched nodal metastases if present. Sections were also stained for vimentin as a possible marker of EMT. The study was funded by Path Soc Leishman Grant 1147.
Results: In this cohort, total ANXA1 expression was not associated with disease progression (p=0.723). A greater proportion of metastatic tumours showed nuclear ANXA1 localisation (40%) compared to non-metastatic invasive tumours (25%) and lepidic-only tumours (22%). Average nuclear expression of nuclear ANXA1 was greater in metastatic tumours and invasive tumours compared to 'lepidic-only' tumours, though this was not a statistically significant result (p=0.301). Nuclear ANXA1 expression was positively correlated with vimentin expression (rho = +0.328, $p<0.05$), suggesting an association with EMT.
Conclusion: These findings, whilst limited by sample size, suggest a possible role for nuclear ANXA1 in driving invasion and metastasis in lung adenocarcinoma, potentially through upregulated EMT.

P20

Immunohistochemically Detectable Metallothionein Expression in Malignant Pleural Mesotheliomas is Strongly Associated with Early Failure to Platin-Based Chemotherapy

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Purpose of the study: Malignant pleural mesothelioma (MPM) is a biologically highly aggressive tumour arising from the pleura with a dismal prognosis. Currently, cisplatin is the drug of choice for the treatment of MPM, and carboplatin seems to have comparable efficacy. Nevertheless, cisplatin treatment results in a response rate of merely 14% and a median survival of less than seven months. Carboplatin resulted in similar response rates ranging from 6 to 16%. Due to their role in many cellular processes, methallothioneins (MTs) have been widely studied in various cancers. The known heavy metal detoxifying effect of MT-I and MT-II may be the reason for heavy metal drug resistance of various cancers including MPM. Methods: 105 patients were retrospectively analysed immunohistochemically for their MT expression levels. Survival analysis was done by Cox-regression (COXPH-model). Cellular response to cisplatin was determined in vitro in association to MT immunoexpression. Additionally, digital miRNA expression as well as methylation analysis of associated loci has been performed.
Summary of results: Cox-regression analyses were done in a linear and logarithmic scale revealing a significant association between expression of MT and shortened overall survival (OS) in a linear (p=0.0009) and logarithmic scale (p=0.0003). Reduced progression free survival (PFS) was also observed for MT expressing tumours (linear: p=0.0134, log: p=0.0152). Conclusion: Since both, overall survival and progression-free survival are negatively correlated with detectable MT expression in MPM, our results indicate a possible resistance to platin-based chemotherapy associated with MT expression upregulation, found exclusively in progressive MPM samples. Initial cell culture studies suggest promoter DNA hypomethylation and expression of miRNA-566 a direct regulator of copper transporter SLC31A1 and a putative regulator of MT1A and MT2A gene expression, to be responsible for the drug resistance

P21

This abstract has been withdrawn

P22

Right-Sided Cardiac Intimal Sarcoma (MDM2 Amplified) with Heterogenous Morphology and Divergent Differentiation

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Background and methods: Cardiac sarcomas are extremely rare primary malignant tumours of the heart. Recent research focuses on cardiac intimal sarcomas arising from the left side of the heart as the most common cardiac sarcomas. This study aims to provide clinico-pathological correlations of the reported cardiac intimal sarcomas exemplified by an unusual case of the right atrial/extracardiac intimal sarcoma with divergent differentiation, diagnosis of which was supported by molecular studies.
Results: 47y.o. man, presented with acute dyspnoea, was found to have large pericardial effusion and a right atrial mass on routine transthoracic echocardiography. Cardiac magnetic resonance imaging showed 59mm mass with heterogeneous enhancement involving the right atrium in connection with 45mm extracardiac mass adherent to inferior cardiac surface in pericardial space. The patient underwent resection of the mass. Several lobulated haemorrhagic and white solid tumour masses were submitted for histological examination, which revealed high grade pleomorphic sarcoma with a variety of morphological features, including foci of floret cell rhabdomyosarcoma with cambian layer and strong nuclear expression of desmin and myogenin. Angiosarcoma was suspected on morphology, but not supported by immunohistochemistry, including ERG expression. CDK4 immunohistochemistry was equivocal and p16 was positive. FISH revealed MDM2 gene amplification at 12q15, supporting the diagnosis of intimal sarcoma. Due to complex involvement of the heart this large tumour was not completely excised. The patient was treated with chemotherapy and is well at 4 months after the diagnosis.
Conclusions: Cardiac sarcomas often present late without overt symptoms, precluding effective tumour eradication. Neoadjuvant therapy targeting MDM2 or PDGFRA if attempted prior to excision may facilitate achievement of complete resection. These rarities should be targeted by 100KGP for better understanding of the pathology.

P23

A Case of Lymphohistiocytoid Malignant Mesothelioma

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A 74 year old male underwent video-assisted thoracoscopic surgery (VATS) for pleural biopsies and talc pleurodesis. The patient had presented with shortness of breath and recurrent right sided pleural effusions on a background of previous asbestos exposure. He was also known to have hypertension, type 2 diabetes and a previous myocardial infarction. At the time of the procedure, no obvious nodules were seen. Histologically, a dense infiltrate of predominantly chronic inflammatory cells comprising CD68 staining histiocytes with a mixed population of CD3 and CD20 positive lymphoid aggregates noted. Admixed amongst these cells, there were clusters and singly dispersed malignant cells with abundant cytoplasm invading the pleura. The malignant cells were positive for calretinin and WT1 and negative for pancytokeratin, TTF1, BerEP4 and CEA. Special stains for fungi and mycobacteria were negative. Lymphohistiocytoid variant of malignant mesothelioma is a rare entity and thought to be a variant of sarcomatoid malignant mesothelioma. The malignant mesothelial cells are characterised by a histocytic appearance admixed with a population of small lymphocytes and occasional plasma cells. Differentials include a Hodgkins and non-Hodgkins lymphoma, lymphoepithelial carcinoma, thymic tumours and reactive lymphoproliferative lesions. The malignant mesothelial cells are often positive for AE1/AE3, calretinin, CK5/6, EMA and negative for CEA, BerEP4, TTF1, CD31, CD34, BCL-2, CD68, CD45 and other lymphocyte markers. Patients typically present with chest pain, fatigue and weight loss. History of asbestos exposure is present in a population of these patients. Patients are usually managed with chemotherapy alone or in combination with radiotherapy. In some cases, palliative surgery with pleurodesis is carried out. Whilst it is a very rare condition, this entity should be considered in any pleural based lesion containing an inflammatory background.

P24

Phosphohistone H3 (PHH3) Immunohistochemical Staining Outperforms Conventional H&E Mitotic Count in Classifying Pulmonary Carcinoids

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Background: Pulmonary carcinoids (PC) are well-differentiated NETs and are classified as typical carcinoid (TC) and atypical carcinoid (AC). Despite the fact that TC and AC exhibit significant differences in patient survival, their classification depends on relatively subtle differences in mitotic count (MC). Although careful counting of mitotic figures (MF) is essential, it is a very subjective task, time-consuming and lacks of sensitivity and interobserver reproducibility, due to selection bias of the hot spots, heterogeneous distribution of MF, difficulty in distinguishing MF from similar chromatin changes (i.e. in apoptotic cells or due to crush, karyorrhectic debris, pyknosis or apoptosis). Identification of MF could be facilitated by the use of mitosis-specific immumostaining such as PHH3.
Methods: 47 PC cases were selected and immunostained for PHH3. MF on both H&E- and PHH3-stained slides were counted in hot spots and per 2 mm2. In tumours that are near the cutoff of 2 per 2 mm2, at least 3 sets of 2 mm2 were counted and the mean used for determining the MC.
Results: Among the 47 PC, 42 cases were TC and 5 were AC when counting MF on HE. 14 of 42 TC (33%) were upgraded to AC and 1 AC was downgraded to TC when MF were evaluated with PHH3. The time needed to count MF on PHH3 stains (~1 minute) was much less than that on HE (~5 minutes). The MC observed on PHH3 was higher as compared with that on H&E. PHH3 stain allows better recognition of mitotic hot spots. The ability of PHH3 to identify hot spots was very useful in heterogenous tumours, avoiding missing AC.
Conclusions: Counting MF with the assistance of PHH3 immunostaining is a more sensitive, more consistent and less time-consuming method for detecting MF than the traditional method of counting MF on H&E.

P25

Variation of von-Willebrand Factor Expression in the Endothelium of Human Coronary Atherosclerotic Plaques: Implications for Thrombosis

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Introduction: Atherosclerotic plaque rupture is often preceded by high macrophagic activity and is complicated by thrombosis: an important mechanism responsible for acute coronary syndrome. Platelet binding to von Willebrand Factor (vWF) is a fundamental mechanism underlying arterial thrombosis. P-selectin and vWF are the principle molecules in Weibel-Palade bodies. Since the former is upregulated during arterial inflammation, this study investigated the changes in vWF expression in various stages of atherogenesis in human tissues.
Hypothesis: Endothelial vWF expression is downregulated over active plaque regions characterised by high macrophagic activity.
Methods: Immunohistochemistry double-staining was performed on paraffin-embedded autopsy specimens of 17 human atherosclerotic coronary artery sections by direct immunoperoxidase for vWF and macrophages. P-selectin and CD31 markers confirmed intact endothelium. The levels of endothelial and sub-endothelial vWF, and intimal CD68+ macrophages were quantified and compared between advanced plaque, active plaque and control regions via image analysis.
Results: In comparison with control regions in the same coronary artery section, vWF intensity of staining was decreased over active plaque regions indicated by high levels of macrophages and P-selectin expression (n=9, p <0.0001). Large, stable plaques (n=5) characterised by low inflammatory cell count, a necrotic lipid core and fibrosis do not follow this trend: vWF expression between plaque and control regions is uniform. Luminal thrombi were negatively correlated with endothelial vWF expression (n=3).
Conclusions: Endothelial vWF expression is dependent on the extent of the inflammatory process underlying the atherosclerotic plaque, acting as a marker for plaque thrombogenicity. Additionally, vWF may be a potential therapeutic target in preventing sudden coronary events, especially in cases of thrombus formation without plaque rupture, as in NSTEMI.

P26

This abstract has been withdrawn

P27

Nine Years Follow Up of a NSCLC Patient with a Novel Germ Line *EGFR* Mutation by Tissue and Liquid Biopsy Based NGS Analyses

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Introduction: Tissue mutation analyses is currently routine diagnostics for targeted treatment of NSCLC. In addition, cell free DNA (cfDNA) analyses (liquid biopsies) are increasingly used in NSCLC patients both for disease monitoring and detection of resistance.
Materials: We report a NSCLC patient with follow-up of 9 years. The patient's lung cytology was investigated by a custom made NGS panel and disease progression and resistance mutations were investigated by NGS with the OncomineTM Lung cfDNA assay. The desired sensitivity in the cfDNA analyses was obtained by combining knowledge of the mutations detected in the cytology material and application of unique molecular identifiers.
Results: In the initial cytology two *EGFR* mutations were identified (V834L: variant allele frequency, VAF 60% and L858R: VAF 56%) and a *TP53* mutation (R248W: VAF 75%). Erlotinib treatment started. Three years later in a lung brush the previously detected mutations were identified again (*EGFR* V834L: VAF 51% and L858R: VAF 9%, *TP53* R248W: VAF 5%). In addition, an *EGFR* T790M (VAF 4%) resistance mutation was detected. Gefitinib treatment followed by osimertinib was applied. During osimertinib treatment 3 longitudinal liquid biopsies were taken. cfDNA analyses identified all previously detected mutations and in addition an *EGFR* C797S resistance mutation. The VAFs of the *EGFR* V834L mutation indicated germ line occurrence. Counselling by Clinical Genetics identified in total 5 family members with an *EGFR* V834L germ line mutation, 4 of them already developed lung cancer all with a somatic *EGFR* L858R mutation.
Conclusion: In a NSCLC patient routine cytology NGS identified a novel germ line *EGFR* mutation. This finding led to genetic counseling and surveillance. The index patient was followed for 9 years and NGS analyses on cytology and cfDNA enabled both disease monitoring and detection of resistance.
Reference: van der Leest et al, *JCO Precis Oncol* (2018), accepted for publication

P28

Consensus Molecular Subtypes Classification of Colorectal Adenomas

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Consensus molecular subtyping (CMS) is an RNA expression-based classification system for colorectal cancer (CRC). Genomic alterations accumulate during CRC pathogenesis, including the premalignant adenoma stage, leading to changes in RNA-expression. Only a minority of adenomas progress to malignancies, which is associated with specific DNA copy number aberrations or microsatellite instability (MSI). This study aimed to investigate whether stratification of colorectal neoplasia into CMS classes can be observed at the adenoma stage, and whether specific CMS classes are related to presence of specific DNA copy number aberrations associated with progression to malignancy. RNA-sequencing was performed on 62 adenomas and 59 CRCs. MSI status was determined by PCR methodology. DNA copy number analysis in adenomas was performed by low-coverage DNA-sequencing (n=30) or array-comparative genomic hybridization (n=32). Adenomas were classified into CMS subtypes with CRCs from the study cohort and from The Cancer Genome Atlas (n=556), using the established CMS classifier. As a result, 54 out of 62 (87%) adenomas were classified according to the CMS. The CMS3 'metabolic subtype', least common among CRCs, was most prevalent among the adenomas (n=45; 72%). One of the two adenomas exhibiting MSI was classified as CMS1 (2%), the 'MSI immune' subtype. Eight adenomas were classified as the 'canonical' CMS2 (13%) type. No adenomas were classified as the 'mesenchymal' CMS4 subtype, consistent with the fact that adenomas lack invasion-associated stroma. The CMS3 class was enriched with adenomas at low risk of progressing to CRC while relatively more high-risk adenomas were observed in the CMS2 subtype. We conclude that adenomas can be stratified into the CMS classes. Considering that CMS1 and CMS2 expression signatures may mark adenomas at increased risk of progression, the distribution of the CMS classes among adenomas is consistent with the proportion of adenomas expected to progress to CRC.

P29

Colorectal Cancer Circulating Tumour DNA Biomarkers: Turning Research into Care

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Purpose of the study: Free circulating tumour DNA (ctDNA) provides promising biomarkers for early diagnosis, prognosis, therapy prediction and disease monitoring of colorectal cancer (CRC) patients. Based on the MEDOCC *Stand Up To Cancer* project and the CAIRO5 phase 3 clinical trial, combined with several other studies, we aim to investigate clinical applicability of ctDNA testing to improve disease management of CRC patients. This requires biomarker validation, assay development, and health technology assessment to determine cost-effectiveness.
Methods: The Prospective Dutch CRC cohort (PLCRC) study is an infrastructure project that arranges informed consent and collects clinical data, tissue and blood samples, and patient reported outcome measures for research purposes. Blood samples are collected in Streck tubes in a multi-centre setting, and sent to a central location (Netherlands Cancer Institute) for isolation of cell-free plasma. Structured clinical, imaging, biobanking, and molecular information is stored in an 'Office Suite' for translational research, offered by the TraIT-Health RI research infrastructure.
Summary of results: Nation-wide collection of clinical data, tissue and plasma samples, molecular data and other features from well-defined large cohorts of stage I-IV CRC patients is currently ongoing. Curated data are stored in the data integration tool tranSMART, allowing data sharing and facilitating health technology assessment.
Conclusions: Our translational research efforts pave the road towards clinical implementation of ctDNA analysis to improve healthcare of CRC patients.

P30

Non-Communicating Isolated Intestinal Duplication Cyst Leading to Mid-Gut Volvulus and Subsequent Acute Intestinal Obstruction: An Unusual Case Report

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Duplication cysts are rare gastrointestinal congenital malformations. They can occur anywhere within the gastrointestinal tract but frequently occur in the small intestine, mainly in the ileum. Duplication cysts have a well-defined muscle coat, an alimentary mucosal lining, and are usually attached to the gastrointestinal tract. They usually share a common blood supply with the native bowel. Completely isolated duplication cysts are an extremely rare variety of gastrointestinal duplications with their own blood supply. There have been a few cases of duplication cyst leading to obstruction, however to the best of our knowledge, this is the first case to report an isolated non-communicating enteric duplication cyst leading to mid-gut volvulus and causing intestinal obstruction.

P31

The Association Between Socio-Economic Status and Colon Cancer Survival By KRAS, BRAF and MSI status: A Molecular Pathology Epidemiology Cohort Study

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Purpose of the study: Previous studies have highlighted socio-economic disparities in colon cancer survival. However, it is unclear if this is due to differences in lifestyle factors, tumour biology or broader issues in healthcare access. To investigate the association between socio-economic status and colon cancer survival by MSI, KRAS and BRAF status, in a large population-based study.
Methods: Northern Ireland (NI) Cancer Registry data was analysed, including all n=1,426 patients who underwent surgery for their Stage II or III colon cancer between 2004 and 2008. Of these, n=661 patients within the NI Biobank jurisdiction were included in molecular analyses. Death information was retrieved via linkage to NI Registrar General's Office up to end 2013. Socio-economic status was derived using the Northern Ireland multiple deprivation measure. The association between socio-economic variables and survival was tested using Cox Proportional Hazard models.
Summary of results: A non-significant increased risk of death were observed for individuals residing in the most compared with the least deprived areas (Most v. least deprived HR 1.21; 95%CI 0.90,1.63). This rose to a significant two-fold increased risk of death in patients with KRAS mutant colorectal tumours (Most v. least deprived HR 2.06; 95%CI 1.25, 3.40), which was not observed for patients KRAS wild-type tumours. Results for survival stratified by MSI and BRAF status remained non-significant.
Conclusions: Colon cancer patients living in more deprived areas of NI had an increased risk of dying, particularly if they had a KRAS mutant tumour, compared with counterparts residing in less deprived areas.

P32

Vitamin D Receptor as a Marker of Prognosis in Oesophageal Adenocarcinoma: A Prospective Cohort Study

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Purpose of the study: Vitamin D receptor (VDR) expression has been associated with survival in several cancer sites. This study aims to evaluate the association between VDR expression and prognosis in oesophageal adenocarcinoma patients.
Methods: There were 130 oesophageal adenocarcinoma resection specimens with clinical data collected from the Northern Ireland Cancer Centre between 2004 and 2012. Tissue microarrays were created and immunohistochemical analysis performed on triplicate tumour cores from each resection specimen. Cox proportional hazards models were applied to evaluate associations between VDR expression and survival outcomes.
Summary of results: During a median of 2.5 (maximum 9) years of follow-up, 75 patients died. In analysis adjusted for confounders, higher VDR expression was associated with an improved overall survival (HR 0.49 95% CI 0.26-0.94) and disease-specific survival (HR 0.50 95% CI 0.26-0.96), when comparing the highest with the lowest tertile of expression. These associations were strongest in sensitivity analysis restricted to junctional tumours.
Conclusions: This study is the first to demonstrate that patients with higher VDR expression in oesophageal adenocarcinoma have a more favourable prognosis. Further work is needed to validate these findings, and to define the role of VDR in the aetiology, progression and management of oesophageal adenocarcinoma.

P33

The Impact of the BSG 2017 Position Statement on Surveillance Workload for Sessile Serrated Lesions will be Limited within Bowel Cancer Screening programme

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Purpose of the study: There is a lack of evidence to inform guidelines for the management of pre-malignant colorectal sessile serrated lesions (SSL). Northern Ireland (NI) is the only UK region where pathologists have diagnosed SSL (or synonyms) during reporting of Bowel Cancer Screening (BCS) specimens since inception of the programme. The aim of this study is to profile SSL diagnoses, and their risk stratification for surveillance, within BCS in NI.
Methods: The NI BCS programme was initiated in 2010, targeting 60-74 year olds through faecal occult blood testing (FOBt). Characteristics of SSL diagnoses were evaluated within the enhanced pathology database completed by all histopathologists reporting BCS specimens in NI. Data up to end March 2017 were considered for analysis, incorporating data on over 17,000 histopathological BCS specimens reviewed at one of four pathology laboratories. Individuals who received a colorectal cancer diagnosis were excluded from analysis.
Summary of Results: SSL were reported in 337 individuals, representing 6.7% of all 5,041 individuals who had a non-malignant histopathological BCS specimen reviewed. One male met criteria for serrated polyposis syndrome, and was excluded from further analysis. Of the remaining 336 SSL cases, 208 (61.9%) were male and 81% had only right-sided SSL detected. Large (≥10mm) SSL or SSL with dysplasia accounted for 82 cases (24.4%), of which only 22 (6.5% of SSL and 0.4% of total 5,041 individuals) did not have concurrent conventional adenomas that would already warrant more frequent surveillance.
Conclusions: SSL cases within this FOBt screening population were predominantly male and right-sided. Of all cases of large or dysplastic SSL, 93.5% also had high or intermediate risk adenomas, which would already require follow-up colonoscopy surveillance at one or three years according to adenoma surveillance guidelines. The clinical significance of concurrent adenomas and SSLs remains to be established.

P34

A Unique Case of Intestinal Sarcoidosis Presenting as Intestinal Obstruction and Mimicking Malignancy

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Intestinal sarcoidosis is an extremely rare manifestation of this multisystem disorder. Gastric sarcoidosis is the most common form of GI sarcoidosis. Symptomatic intestinal involvement is rare, with only a few cases described in the literature showing well documented histological evidence of non-caseating granulomas. We present a unique case of intestinal sarcoidosis presenting with obstruction in a 58 year old female with a history of myeloid leukaemia and sarcoidosis. Radiology revealed a closed loop large bowel obstruction with a 60 mm long stricture in the proximal descending colon which was suspected as malignant. The patient underwent a left hemicolectomy with a colostomy. Macroscopically the lumen of large bowel was occluded by a stricture filling the lumen with a cream coloured firm cut surface. Microscopy showed heavy mixed inflammatory infiltrate composed of lymphocytes, histiocytes, plasma cells with neutrophils and occasional eosinophils with interspersed non-caseating granulomas. Symptomatic involvement of gastrointestinal sarcoidosis is extremely rare. This case highlights the gastrointestinal involvement of this multisystemic disorder with unusually rare presentation of intestinal obstruction mimicking a malignancy.

P35

The Effect of T-Regulatory Lymphocytes on Patient Survival Following Neoadjuvant Chemotherapy for Oesophageal Adenocarcinoma

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Purpose of the study: Oesophageal cancer carries a poor survival of 12.3% at five years. Its predominant histological subtype in the Western world, oesophageal adenocarcinoma, is increasing in incidence. The influence that the host inflammatory response has on tumour progression and ultimately patient survival is being increasingly understood. Previously it has been found that the Klintrup-Makinen criteria can be used to grade the overall local inflammatory response in oesophageal adenocarcinoma to stratify patients into prognostic groups. Detailed assessment of the cellular components of the local inflammatory infiltrate may provide insight into the driving force of its prognostic value. In this study we aim to investigate the prognostic effect of FOXP3+ T-regulatory lymphocyte infiltration in a homogenous cohort of patients with oesophageal adenocarcinoma treated with neoadjuvant chemotherapy and surgical resection.
Methods: Included in the study were patients with oesophageal adenocarcinoma who were treated with neoadjuvant chemotherapy and surgical resection with curative intent. Immunohistochemistry was used to assess FOXP3+ T-regulatory lymphocyte infiltration.
Summary of results: Tissue was available for 72 patients for analysis. The median survival for FOXP3+^{low} patients was 29.8 (95% CI 11.4-48.2) months versus 22.3 (95% CI 16.1-28.5) months for FOXP3+^{high} patients (p=0.096). Lower FOXP3+ infiltrate was significantly associated with higher Klintrup-Makinen score (p=0.003).
Conclusions: The present study demonstrates that FOXP3+ T-regulatory lymphocyte infiltrate does not predict survival in a homogenous cohort of patients with oesophageal adenocarcinoma treated with neoadjuvant chemotherapy and surgical resection with curative intent. However, it does demonstrate a significant negative association between FOXP3+ T-regulatory lymphocyte infiltrate and Klintrup-Makinen score.

P36

RNF43 Frameshift Mutations Contributes to Tumourigenesis in Right Colon Cancer

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RNF43 (Ring finger protein 43) is a single transmembrane ring-type E3 ubiquitin ligases and an important negative regulator of the Wnt signal pathway. Truncating mutations of RNF43 frequently occurred in colorectal cancer (CRC). In this study, we systematically analyzed RNF43 frameshift mutation R117.fs and G659.fs in 2094 CRC samples from our tissue bank, the TCGA database, the DFCI_2016 and MSK_2018 dataset. Our study showed that RNF43 frameshift mutation was correlated with MSI-H (OR=170.16, 95% CI=23.64-1224.59, I2=67%), BRAF V600E mutation (OR=7.67, 95% CI= 2.68-21.98, I2=84%), distant metastasis (OR=0.32, 95% CI=0.17-0.57, I2=35%), advanced stage (OR=0.35, 95% CI=0.22-0.53, I2=16%)and CRC located in the right colon (OR=6.56, 95% CI = 2.03-21.15, I2=82%). After adjusting for MSI status, there is no longer a correlation between RNF43 frameshift mutation and distant metastasis (OR=1.89, 95% CI=0.83 - 3.87, I2=0) or advanced TNM stages (OR=0.97, 95% CI=0.56- 1.69, I2=7%). However, there remains a correlation between RNF43 frameshift mutations and right-sided CRC (OR=2.63, 95% CI=1.40-4.94, I2=0) and BRAF V600E mutation (OR=1.87, 95% CI = 1.11-3.13, I2=64%). In conclusion, RNF43 frameshift mutations in CRC were related to distant metastasis and TNM-stage depending on MSI status, but contributed to tumourigenesis in right colon cancer independent of MSI status.

P37

Time to Get Off the Fence? Colorectal Biopsy Diagnosis of "Suspicious for Adenocarcinoma" has a 100% Positive Predictive Value for Malignancy on Resection

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Purpose of the study: To determine the predictive value for malignancy of "suspicious for adenocarcinoma" in colorectal biopsy reports and to detect subgroups in which a definitive diagnosis of adenocarcinoma can be confidently made.
Methods: A SNOMED search of our departmental database was undertaken for colorectal specimens processed between 2014 and 2016. The pathology reports were reviewed and colorectal biopsies were identified which had been reported as "suspicious for adenocarcinoma". Further data were extracted from the reports, and where available correlation was undertaken with the resection specimens.
Results: 4996 specimens were identified, of which 86 biopsies (from 82 patients) were reported as "suspicious for adenocarcinoma". The mean age was 74, with a male : female ratio of 1.65 : 1. The mean number of fragments submitted was 5. 37% of the biopsies were rectal. 64% of patients underwent resection, of which 100% contained adenocarcinoma. 8% were poorly differentiated and 71% were stage pT3/T4. On review of clinical information provided, 'tumour' was described in 51% and 'looks malignant', 'stricturing', 'obstructing' or 'circumferential' in 31%. Stromal desmoplasia was described in 16% of microscopic reports. Of those with a combination of 'tumour' in clinical details and reported stromal desmoplasia, 75% underwent resection, all of which contained cancer.
Conclusions: Colorectal biopsies reported as "suspicious for adenocarcinoma" have a 100% positive predictive value for adenocarcinoma on resection. There were no false positives in our series. Patients for whom no resection specimen was available may have been unfit for surgery, may have had pM1 disease at the time of diagnosis or may have had surgery at another institution. Patients with a clinically described "tumour" with microscopically-identified stromal desmoplasia had a high resection rate and pathologists should consider making a confident biopsy diagnosis of malignancy in this context.

P38

Comparison of Mismatch Repair Protein Expression Between Biopsies and Resections in Advanced Colon Cancer from the Phase II Group of the Fluoropyrimidine Oxaliplatin and Targeted Receptor Pre-Operative Therapy (FOxTROT) Trial

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Colorectal cancer is diagnosed in around 41,000 people a year in the UK. Approximately 15% develop following a defect in the mismatch repair (MMR) pathway. FOxTROT is a novel trial assessing the potential benefit of pre-operative chemotherapy in locally advanced colon cancer. We aimed to compare MMR status between biopsy and resection tissue from the phase II component of the FOxTROT trial and identify possible changes following chemotherapy. Cases of complete/near complete response were not tested (n=8). Resection material for 131 cases in tissue microarrays (3 tumour cores per case) was stained using immunohistochemistry for MLH1, PMS2, MSH2 and MSH6. 100 cases had matched biopsy material. Slides were digitally scanned and scored as either positive or negative by two independent pathologists. In cases with poor staining or discordant scoring, repeat staining was performed on whole tissue sections. ***Part funded by PathSoc.***
99 cases showed proficient MMR with the remainder (24%) showing deficiency. Of the deficient cases, the majority showed loss of MLH1/PMS2 (n=25) with loss of MSH2/MSH6 in 2 cases, isolated PMS2 loss in 2 cases and isolated MSH6 loss in 1 case. 1 case showed additional clonal negativity of its non-paired protein. The remaining 2 cases showed unusual patterns of loss of one or more proteins. Of the 25 cases with MLH1/PMS2 loss, 15 were associated with a BRAF codon 600 mutation and 10 were wild type. There was agreement in MMR status between the biopsy and resection in 98% of cases (n=98). The two discordant cases showed unusual patterns of sub-clonal protein loss not entirely consistent across the biopsy and resection. There was a very good correlation in MMR protein expression status between the biopsy and resection in the FOxTROT trial. The two discrepant cases are currently undergoing further investigation. There is no evidence that pre-operative chemotherapy induces a change in MMR status unlike the loss of MSH6 described following radiotherapy.

P39

Lynch Syndrome Screening Across a Population of 5.7 Million Through a Regional Bowel Cancer Improvement Programme: Results of the First 829 Cases

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Lynch syndrome (LS) is implicated in around 3% of colorectal cancers (CRC). Recent NICE guidance recommends screening all newly diagnosed CRC for LS so that patients and their families can be managed appropriately. A regional Bowel Cancer Improvement Programme (BCIP) aims to improve outcomes for patients with CRC across a population of 5.7 million, and includes initial funding for a new regional LS screening programme. All newly diagnosed CRC over the age of 50 years across the 16 multidisciplinary teams in the region were eligible for LS screening through the BCIP, starting in May 2017. Cases diagnosed under 50 years were expected to follow routine pathways in place following previous guidance. Screening consisted of immunohistochemistry for MLH1, PMS2, MSH2, and MSH6 supported by BRAF codon 600 analysis by pyrosequencing where appropriate. MLH1 promoter hypermethylation analysis has recently been developed. Samples from 906 patients were received from ten hospitals by 1st March 2018, with results available in 829. 516 were tested on biopsies/polyps and 313 on resections. 94 cases showed deficient mismatch repair (11%) of which 71 showed loss of MLH1/PMS2, 6 loss of PMS2, 9 loss of MSH2/MSH6 and 3 loss of MSH6. In addition, 3 cases showed patchy loss of one or more proteins, 1 case showed PMS2/MSH6 loss and 1 case showed PMS2 loss with patchy MLH1 loss. Additional heterogeneous/subclonal loss of a non-paired protein was seen in 9 cases. For cases with MLH1 loss, 48 showed a mutation in BRAF and 20 were wild type, of which 4 showed hypermethylation with the remainder awaiting testing. BRAF failed for 3 cases. Through the regional BCIP, we have established one of the largest regional LS screening programmes in the UK with 829 cases screened to date. The programme has detected 20 patients (2.4%) with possible LS who have been recommended for genetic counselling and consideration of germline testing.

P40

An Audit of the Quality of Pathology Reporting for Colorectal Cancer Resections Across a Population of 5.7 Million Through the Regional Bowel Cancer Improvement Programme

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Colorectal cancer (CRC) is the second most common cause of cancer related mortality in the UK. There is significant variation in management and outcomes nationally. The Bowel Cancer Improvement Programme (BCIP) is a regional 5 year study aiming to standardise practice across surgery, pathology, radiology and oncology to improve outcomes across a population of 5.7 million. We aimed to audit the quality of CRC resection pathology reporting in the 16 regional hospitals against the Royal College of Pathologists (RCPath) Dataset. 48 pathologists from 9 hospitals participated by submitting 10 consecutive pre-2018 CRC resection pathology reports, anonymised for both patient and pathologist details. Reports on local excisions, neuroendocrine tumours, and those with no cancer (except complete response to neoadjuvant therapy) were excluded (n=7). A total of 473 reports were audited against the 3rd edition RCPath Dataset for CRC. Overall, macroscopic "core" items were reported in over 95%. The majority of microscopic "core" items such as: tumour type, differentiation and pT stage were included in all reports, however, distance to the circumferential resection margin (CRM) was only reported in 72%. In rectal cancers (n=133), the relationship of tumour to the peritoneal reflection was reported in 99% and the mesorectal grade, distance to dentate line and sphincteric grade in 91%, where applicable. Macroscopic "non-core" items were given in the majority of cases where relevant except mesocolic grade (11%). Microscopic "non-core" items including tumour budding and perineural invasion were infrequently given with the exception of lymphatic invasion (34%).Pathologists across the region are generally excellent at including "core" macroscopic and microscopic items in pathology reports following CRC resection, however, there are some areas that require improvement. Education on the importance of a complete dataset is planned and we will re-audit against the 4th edition later this year.

P41

Total/Clonal Null Phenotype Mismatch Repair Protein Expression During Lynch Syndrome Screening in the Regional Bowel Cancer Improvement Programme: An Unusual Series of Cases

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Approximately 3% of colorectal cancers (CRC) are associated with a germline mutation in the mismatch repair (MMR) pathway characteristic of Lynch syndrome (LS). NICE recommends screening for LS in all cases of newly diagnosed CRC. Unusual patterns of staining with MMR immunohistochemistry (IHC) have previously been described. The Bowel Cancer Improvement Programme hosts a regional LS screening programme across a population of 5.7 million for all new CRC diagnoses over 50 years . We aimed to investigate cases with unusual patterns of deficient MMR. Since May 2017, 829 patients have been screened using IHC for MLH1, PMS2, MSH2 and MSH6, followed by pyrosequencing of BRAF codon 600 where appropriate. Four cases with total or clonal null phenotype were identified. The resection specimen was tested in 3 cases and the biopsy in the remaining case. If available, multiple tumour blocks were independently stained. All 4 cases occurred in elderly patients (mean 80 years) with right sided colon tumours. None had undergone preoperative treatment. All cases showed complete loss of MLH1 and PMS2 with a BRAF codon 600 mutation, in keeping with sporadic deficient MMR. In addition, one biopsy showed total loss of MSH2/MSH6; two resections showed clonal loss of MSH2/MSH6; and one resection showed two distinct sub-clones, one with loss of MSH2/MSH6 and one with loss of MSH6 alone. Two cases are currently undergoing further testing. Cases with a null phenotype with MMR IHC have been described but are unusual. The loss of MLH1/PMS2 with a BRAF mutation and co-existent clonal loss of MSH2 and MHS6 is most likely due to further sporadic double hit mutations, however, with total absence of expression a germline mutation cannot be entirely excluded. Further investigation including sequencing of different clonal areas for MMR gene mutations is currently being performed to confirm our suspicions that these are further somatic events and prevent unnecessary referrals for germline testing.

P42

Predicting the Response to Radiotherapy in Rectal Cancer: Results of a Pilot Study Based on Macrophage Subpopulations

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Over 41,000 new cases of colorectal cancer occur in the UK every year. Many patients with rectal cancer will undergo neoadjuvant radiotherapy that can cause significant long term morbidity. There are currently no validated predictive biomarkers to enable selection of only those more likely to receive oncological benefit. Macrophage subpopulations within the tumour microenvironment influence tumour behaviour. We aimed to determine whether macrophage subpopulations are predictive of response to radiotherapy in rectal cancer. 52 rectal cancer patients treated with short course radiotherapy followed by surgery at a large UK radiotherapy centre were identified. Immunohistochemical staining was performed on the formalin fixed paraffin embedded biopsy material using HLA-DR and CD163 antibodies (markers for M1 and M2 macrophages respectively). Dual staining for each marker was performed with CD68 to ensure staining was macrophage specific. Staining was measured objectively in the three 1.2 mm diameter circles of greatest staining using pixel counts via Nuance Multispectrum Imaging, and classified as either low or high by the median value. Tumour response was measured by the percentage of residual tumour cell density (TCD) in the post-treatment resection when compared to the pre-treatment biopsy. Patients with low CD163-CD68 co-localisation had a median residual TCD of 30.19% (IQR 12.09-86.70) whereas those with high co-localisation had a median residual TCD of 50.73%. (IQR 18.17-74.59, p=0.62). Patients with low HLA-DR-CD68 co-localisation had a median residual TCD of 30.19% (IQR 12.09-55.66) whereas those with high co-localisation had a median residual TCD of 53.83% (IQR 14.60-86.70 p=0.18). In this pilot study, macrophage subpopulations in the tumour microenvironment were found not to be predictive of response to radiotherapy. To understand the role of the immune system in radiotherapy response, further studies are needed looking at a wider panel of immune cell markers.

P43

Following Tumour Biology at the Single Cell Level Using Microfluidics in Colorectal Cancer: A Potential Role for Guiding Personalised Patient Treatment?

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Approximately 42,000 new cases of colorectal cancer are diagnosed in the UK annually. Patients may undergo pre-operative radiotherapy, chemotherapy or both. Analysis of tumour biology at the single cell level could help to achieve better patient outcomes by assessing the response to pre-operative therapy in different subclones and guiding treatment. We aimed to assess the sensitivity of cell lines to different treatments before growing in spheroids and analysing using microfluidics. HCT-116 and HT-29 cell lines were maintained as monolayer cultures in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% Fetal Bovine Serum, 2mM Glutamax and Penicillin 100units/mL Streptomycin 100ug/mL. The alamarBlue viability assay was used to measure the dose response of cells subjected to treatment of common colorectal cancer drugs, 5-flurouracil, irinotecan and oxaliplatin. Fluorescence was read with an excitation wavelength of 570nm and emission wavelength of 590nm. HCT-116 and HT-29 cell lines were cultured in 3D using epiFL, a modified form of FibroLife medium. Cell line sensitivity of the drugs was determined by calculating the IC50 values.
The IC50 values when HCT-116 cells were subjected to 5-flurouracil, irinotecan and oxaliplatin treatment were 0.65µg/mL, 9.48 µg/mL and 1.8 µg/mL respectively. When HT-29 cells were subjected to the same drugs, the IC50 values were 3.26 µg/mL, 14.49 µg/mL and 1.93 µg/mL respectively. Both HCT-116 and HT-29 cell lines successfully formed tight spheroids ranging in diameter from ~150-250µm. We have successfully grown colorectal cancer cell lines as both monolayers and spheroids, and determined sensitivity to common colorectal cancer drugs. Ongoing work will now take these experiments to the micro-scale and assess the practicality of using microfluidic devices to trap single cells from these spheroids and analyse the response to treatment at the single cell level.

P44

Is Whole Slide Imaging (WSI) Non-Inferior to Conventional Light Microscopy (CLM) in the Assessment of Specimens from the UK Bowel Cancer Screening Programme?

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Background: The diagnostic pathology workload in the UK is increasing in volume and complexity, and many departments are considering the adoption of digital pathology to accommodate for this. Prior to implementing whole slide imaging (WSI) it is crucial to be certain that it will not alter a pathologist's interpretation of screening programme biopsies.
Aims: The primary aim of this study was to investigate the intraobserver variation between pathologists' diagnoses of bowel polyps on conventional light microscopy (CLM) and WSI platforms. Secondary aims were to compare the assessment of size of polyps, the pathologists' diagnostic confidence and the times taken to reach a diagnosis on both platforms.
Methods: Four pathologists were recruited to diagnose 50 single case polyp slides from the bowel cancer screening programme. For 10 of these cases the pathologists were also required to measure the polyp length. The pathologists viewed each case 4 times, twice using CLM and twice using WSI. Diagnostic data and confidence metrics were collected using a tick box pro forma and the time taken to reach a diagnosis was recorded.
Results: On CLM versus WSI the intraobserver variation for the classification of polyps and dysplasia levels were almost perfect for pathologists A, B and C; kappa = 0.92, 0.86, 0.81 and 0.95, 0.92, 0.87 respectively. For pathologist D the concordance was moderate, kappa = 0.51 when classifying polyps and substantial, kappa = 0.68, in the assessment of dysplasia. The median time taken for the pathologists to reach a diagnosis was 53 seconds on CLM and 55 seconds on WSI ($t = 2.343$, $p = 0.02$).
Conclusion: WSI technology has the potential to transform the way in which screening programme diagnostics are reported. This study found an excellent concordance between diagnoses of bowel polyps on CLM and WSI. Nonetheless, it is important that pathologists train appropriately in the use of digital pathology to ensure competent and confident diagnoses are made.

P45

Somatic Mutations of Beta2-Microglobulin are Correlated with a Lack of Disease Recurrence in a Subset of Stage II Mismatch Repair Deficient Colorectal Cancers from the QUASAR Trial

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Introduction: Cell-surface antigen presentation is reliant upon a functional Beta2-Microglobulin (B2M) - HLA class I complex. Due to the presence of many microsatellites, *B2M* is prone to mutations in mismatch repair-deficient colorectal cancer (dMMR CRC) and limited data suggest that this may afford protect against disease recurrence. Here, we examined *B2M* mutation status, B2M protein expression and immune infiltration in relation to recurrence in patients enrolled in the stage II QUASAR trial.
Methods: Mutation status of *B2M* was determined by Sanger sequencing on 121 dMMR and 108 proficient mismatch repair (pMMR) tumours, 52 (48.1%) with recurrence and 56 without. Protein expression was assessed by immunohistochemistry and immune infiltration assessed by H&E review. Mutation status and protein expression were correlated with recurrence and compared to pMMR CRCs.
Results: 32% (39/121) dMMR CRCs showed pathogenic *B2M* mutations. None of these *B2M*-mutant tumours recurred (median follow-up 7.4 years), however 18% (14/77) *B2M*-wildtype tumours did (p=0.005); six locally and eight at distant sites. Sensitivity and specificity of IHC in detecting *B2M* mutations was 87% and 71% respectively. Significantly fewer 3/104 (2.9%) of the 108 pMMR CRCs demonstrated deleterious *B2M* mutations (p<0.0001). No difference was seen in the amount of immune infiltration between the *B2M*-mutant and *B2M*-wildtype tumours, with approximately 20% of each group displaying low level infiltration. A pathogenic mutation was detected in a single pMMR tumour which recurred.
Discussion: *B2M* mutations were detected in nearly one third of dMMR cancers, none of which recurred, providing evidence that *B2M* mutation status has potential prognostic utility in a subset of stage II dMMR CRC, although the mechanism remains to be proven in this setting.

P46

Luminal Tumour Morphometry Predicts Poor Prognosis in Patients with Intestinal Type Gastric Cancer

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Purpose of study: A significant proportion of gastric cancer (GC) patients with locally advanced (stage II/III) tumours have recurrent disease despite attempted curative resection. At present there is no means to identify these high risk patients. As tumour morphometry has proven prognostic value in other tumour types, we hypothesised that the proportion of tumour (PoT) measured at the luminal surface (and thus measurable in endoscopic biopsies) predicts GC patient survival and can thus identify high risk patients at the time of diagnostic biopsy.
Methods: Luminal PoT was measured by digital morphometry in 231 stage II/III GC resection specimens obtained from the Kanagawa Cancer Center, Yokohama, Japan. Tissue microarrays were used to assess the extent of CD45 positive immune cell infiltration. Results were related to histopathological features and patient overall survival (OS).
Summary of results: PoT was significantly lower in diffuse-type GC when compared to intestinal-type GC (30% vs. 41%, p=0.03). Patients with low PoT intestinal-type GC survived significantly longer than patients with high PoT intestinal-type GC (5 year OS = 78% vs 47%, p=0.011). Low PoT was independently prognostic in multivariate analyses in intestinal-type GC. Low PoT additionally correlated with high CD45 positive immune cell infiltration (p=0.035). There was no association between PoT and OS in diffuse-type GC nor when analysed across the entire patient cohort.
Conclusions: We have identified a novel subgroup of stage II/III intestinal-type GC patients at high risk of mortality by measuring PoT at the luminal surface. The relationship between PoT and immune cell content is suggestive of a potential underlying mechanism. Potential clinical value warrants validation in a second GC cohort.

P47

A Rare Cause of Ascites in the Immunocompromised Patient

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Primary effusion lymphoma (PEL) is an aggressive, rare, large B-cell neoplasm, usually seen in the context of immunodeficiency and almost universally associated with human herpesvirus type 8 (HHV-8). Classically, it involves body cavities with no detectable, solid tumour mass and possesses a distinctive immunophenotype. We report the case of a middle-aged patient developing ascites during treatment for Kaposi's sarcoma. They had previously been treated for suspected peritoneal tuberculosis and were known to be HIV-positive for almost a decade with poor concordance to anti-retroviral therapy. As part of the diagnostic workup, a sample was sent for cytology which demonstrated dispersed atypical cells with enlarged, pleomorphic nuclei with variable lobation and nucleolation. On immunohistochemistry, the atypical cells stained positive for HHV-8, CD3, CD43, CD138 and MUM-1 and were negative for PAX5. EBER-ISH was negative. Clonality was confirmed with receptor rearrangement studies. These features were diagnostic of a HHV-8 positive primary effusion lymphoma and the patient was commenced on systemic chemotherapy. This case provides a good illustration to cytopathologists of a seldom seen cause of a lymphocytic cavity effusion. In order to recognise this entity and use ancillary testing judiciously on a clot preparation with limited material, it is important to know the clinical history and remember that, despite being a B-cell neoplasm, this entity can lack pan B-cell markers and show expression of some T-cell markers. The prognosis remains very poor although prompt diagnosis and initiation of chemotherapy and correction of the immunosuppression can confer a small survival benefit.

P48

Multiple Myeloma in a 6 Year Old Child: A Case Report

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Multiple myeloma is a haematological malignancy characterised by proliferation of clonal plasma cells in the bone marrow. Multiple myeloma in children is extremely rare with only a very few reported cases in the literature. A 6 year old girl presented with a short history of back pain followed by bladder/bowel dysfunction. She was found to have a large thoracic extradural space occupying lesion. Debulking of the tumour revealed fragments of bone and connective tissue extensively infiltrated by a tumour composed of solid sheets of cells with plasmacytoid morphology. The tumour showed marked nuclear pleomorphism with high mitotic activity. Immunohistochemistry showed the tumour was positive for plasma cell markers CD138 and MUM1 with aberrant expression of cyclin D1 and prominent lambda light chain restriction. PCR showed clonal rearrangement of the immunoglobulin heavy and light chains. Further investigations revealed an abnormal serum free light chain ratio with normal immunoglobulin levels and no evidence of an M protein. A bilateral bone marrow trephine showed an infiltrate of atypical plasma cells which exhibited prominent lambda light chain restriction. An MRI scan revealed multiple lytic lesions with no evidence of nodal or solid organ involvement. Following correlation of the histological findings with other investigations, the features were consistent with a plasma cell myeloma. Plasma cell myeloma is extremely rare in children with fewer than 10 cases described in the literature in patients younger than 10 years. To our knowledge, this is the first case described in an immunocompetent patient at such a young age.

P49

The Use of Flow Cytometry in the Assessment of Tonsil Specimens

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Introduction: Flow cytometry (FC) is now routinely used as a further diagnostic tool in the evaluation of haematological malignancies, including leukaemia and lymphoma. In this retrospective study, we evaluated the diagnostic use of flow cytometry in tonsil biopsies and tonsillectomy specimens.
Methods: We reviewed all tonsil biopsies/tonsillectomy specimens reported by Haematopathologists in our department between 2012 and 2017. In cases where flow cytometry was carried out, results were correlated with the histopathology findings.
Results: 88 cases of tonsil biopsies/tonsillectomy specimens were found and reviewed. There were 47 males and 41 females with a mean age of 26.5 (2.5 – 81.1). FC was carried out in 37 cases. In 34 cases (92%), FC did not show an abnormal population of lymphoid cells. Of these 34 cases, 3 were found on histology to have a B cell lymphoma (2 DLBCL, 1 early post-transplant lymphoproliferative disorder). In 3 cases (8%), FC identified an abnormal population of B cells which, on subsequent histology, were found to have a B cell lymphoma (DLBCL, Follicular lymphoma, Mantle cell lymphoma). Most cases with a histological diagnosis of lymphoma/lymphoproliferative disorder occurred in adults with a mean age of 56.2 (range 4.8 – 81.1).
Conclusion: Tonsillar haematolymphoid malignancy is uncommon, in particular in the younger age groups. Our findings would suggest flow cytometry is of limited use as an ancillary technique in the evaluation of haematological malignancies at this site.

P50

Epstein Barr Virus Latent Membrane Protein 1 in Lymphoma in a Tertiary Hospital in Ghana

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Purpose of the study: Epstein-Barr virus (EBV) genome and encoded antigens has been isolated from Hodgkin and Non Hodgkin lymphoma by immunohistochemistry (IHC) through the detection of EBV latent membrane protein 1 (LMP-1). The aim of this study is to determine the presence of EBV LMP-1 in lymphoma cases in our centre.
Methods: We randomly selected 60 cases of Lymphoma and their clinical demography from our archive, retrieved their corresponding FFPE blocks and performed IHC on them through the 2 step peroxidase anti-peroxidase technique with a limited panel of antibody comprising of CD45, 30, 20, 3 and EBV LMP-1.
Results: The age range of cases was 2-77 years; mean age 22.47 years. Thirty nine cases (69.5%) were in the first and second decade while 8 cases (13.3%) were in the sixth to ninth decade. The male to female ratio was equal. Histological diagnosis shows that Hodgkin lymphomas were 13 cases (21.7%) and Non Hodgkin Lymphoma were 47 cases (78.3%). Out of the NHL, 7 cases were of T cell lineage (positive for CD3) and 30 cases were of B cell lineage (positive for CD20) and 10 cases were indeterminate. EBV LMP-1 was expressed in 19 cases (31.7%). Six (46.2%) out 13 cases of HL were positive while 13 cases (27.7 %) out of 47 NHL cases were positive.
Conclusions: This study lends credence to the role of EBV in lymphoma through the expression of EBV LMP-1 in our centre.

P51

An Audit of Low Grade B Cell Lymphomas of the Gastrointestinal Tract

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Introduction: The gastrointestinal tract is the most common extranodal site involved by lymphoma. The majority of gastrointestinal lymphomas are of B cell origin. Low grade B cell lymphomas represent a significant proportion of the lymphomas diagnosed within the gastrointestinal tract. They represent a diagnostic challenge due to their variable and subtle features. Their insidious nature and possibility of transformation means that appropriate follow up is of great importance.
Methods: We audited the cases of low grade B cell lymphomas diagnosed within the gastrointestinal tract over a two year period within a tertiary referral centre. The immunoprofile of each case and clinical behaviour was examined.
Results: 46 cases were identified, these comprised 35 extranodal marginal zone lymphoma (MALT lymphoma), 7 follicular lymphoma, 4 mantle cell lymphoma and 1 chronic lymphocytic leukaemia.
Conclusion: We have reviewed the rate of diagnosis, immunoprofile and clinical progression of 46 cases of low grade B cell lymphomas diagnosed within the gastrointestinal tract. We have validated the use of recognised immunohistochemical markers in the diagnosis of each subtype, and the merit of follow up at recommended intervals.

P52

Granulomatous Slack Skin/Granulomatous Mycosis Fungoides: A Rare Variant of Cutaneous T Cell Lymphoma

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Granulomatous slack skin (GSS) and granulomatous mycosis fungoides (GMF) are recognised rare variants of cutaneous T cell lymphomas (CTCL) with overlapping histological features. We present a case of a 70 year old female with recurrent cutaneous lesions in a background of known GSS diagnosed in 2012. She received radiotherapy as treatment for the initial lesion. She subsequently developed nodal Grade 3a follicular lymphoma in 2014 and mixed cell Hodgkin lymphoma in 2015 which required chemotherapy and is currently in remission for these conditions. Incidental hilar nodes found on follow up CT scan and abnormal liver function tests led to a fourth diagnosis of mediastinal and hepatic sarcoidosis in 2015, which did not require treatment. She presented with new abdominal lesions and biopsy confirmed granulomatous cutaneous T cell lymphoma with the same T cell clones as the previous biopsies. No obvious epidermotropism was seen but there were sarcoid-like as well as more diffuse granulomas present. A diagnosis of granulomatous mycosis fungoides was made. Patients with granulomatous CTCLs are known to be at risk of developing second lymphoid neoplasms and unusually, in our case, the patient developed two distinct lymphoid neoplasms after the initial diagnosis. GMF and GSS have similar histological features and are mainly distinguished by their clinical appearances. In most instances there is no or little epidermotropism, unlike classical mycosis fungoides. Although rare, it is an entity to be aware of in granulomatous inflammation of the skin.

P53

Megakaryocyte Clustering on Bone Marrow Trephine of a Non-Sclerotic Area in a Lady with POEMS Syndrome: A Case Report

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POEMS syndrome is a rare variant of osteosclerotic myeloma which causes multiorgan dysfunction. Reported incidence is three per million, but as awareness of the condition is increasing, the figure is expected to rise, as many cases may be mistaken as chronic inflammatory demyelinating polyneuropathy (CIDP). The pathogenesis is unclear, but the production of lambda light chains by a collection of dyscrasic plasma cells and subsequent auto-immune attack has been postulated as the cause of symptoms. Vasoendothelial growth factor (VEGF) has also been suspected as a pathogenic agent, as levels are commonly raised. Osteosclerotic lesions are typical, and if biopsied, demonstrate plasma cell aggregation which will highlight on lambda light chain restriction.

P54

Inflammasomes in Healthy and Demyelinated CNS

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Purpose of the study: Damaged tissue and foreign material pose a threat to our body. The innate immune system can recognise these 'danger signals' and form the inflammasome. This releases pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18) out of the cell which activate the immune response against the danger. Inflammasomes are thought to be involved in multiple sclerosis (MS). MS is a chronic demyelinating disease that causes neurological disability. Mixed glial cultures, experimental autoimmune encephalomyelitis (EAE) and lysolecithin are models used to study MS pathogenesis. We hypothesise that inflammasomes are involved in both CNS maintenance and pathology.
Methods: Spinal cords sections from healthy, EAE- and lysolecithin-demyelinated WT mice were stained to assess the inflammasome expression profile. Mixed glial cultures were generated from neonatal mouse brains. Cells were seeded in 96-well plates and were stimulated either with inflammasome mediators, IL-1β and IL-18, to test glial response to inflammasome activation, or with inflammasome activators to test endogenous inflammasome response in glial cells.
Summary of results: Inflammasome markers are expressed in healthy CNS and accumulate within demyelinated lesions in diseased tissue in animal models of MS. The inflammasome is activated in mixed glial cells upon stimulation, indicating that glial cells can react to danger signals. In addition, glial cells respond to inflammasome activity via enhanced differentiation.
Conclusion: We conclude that inflammasomes and their products are expressed in the healthy CNS and might be involved in demyelination pathogenesis. Our results suggest a new role of inflammasomes in glial cells, demonstrating that they can react to both danger signals via inflammasome activation and to inflammasome mediators. Future work will uncover the mechanisms underlying inflammasome-mediated glial cell differentiation.

P55

The Monocle Tumour: A Diagnostic Challenge

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The monocle tumour is a primary histiocytoid carcinoma of the periocular region. Morphologically similar, histiocytoid breast carcinoma has a predilection for orbital metastases. It is currently not possible to discriminate between these two lesions using immunohistochemical or molecular markers. This means it can be difficult for the clinician and pathologist to make the correct diagnosis. An incorrect diagnosis can have management implications. Herein, we present two cases to help illustrate the challenges of diagnosing periocular histiocytoid carcinoma. The first case is an example of primary periocular histiocytoid carcinoma, whereas the second case is an example of metastatic histiocytoid breast carcinoma. Both cases were stained for a variety of immunohistochemical markers and these findings are presented with the relevant clinical history and imaging. To the best of our knowledge, we are the first to show that GATA-3 can be expressed in primary periocular histiocytoid carcinoma.

P56

Neuropathology Elective at Johns Hopkins Hospital: A Personal Account

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Aims: (1) To gain clinical exposure to neuropathology by shadowing neuropathologists within the department; (2) To conduct neuropathological research that may lead to publication; and (3) To develop an understanding of the US healthcare system.
Results: My clinical exposure was varied and interesting. I shadowed several neuropathologists and learnt about their day-to-day routines, roles and responsibilities within the department. By the end of the elective, I could recognise normal brain tissue, differentiate between most cell types, and accurately diagnose some of the more common brain tumours or neurodegenerative diseases. I also learnt how to count mitoses, and grade tumours such as gliomas, and developed an appreciation for the different immunohistochemical stains or molecular testing that could aid diagnosis and inform management. My research project consisted of performing DNA mutational analysis on low grade gliomas. I learnt how to process DNA for sequencing as well as how to analyse the results.
Conclusion: Undertaking an 8-week elective at Johns Hopkins Hospital, USA, provided me with valuable insight into a career in neuropathology. For any medical student interested in pathology, I would highly recommend completing an elective or clinical attachment in this speciality.
I would like to take this opportunity to thank the Pathological Society for providing me with a generous £1200 undergraduate bursary. My elective would not have been possible without their support for which I am truly grateful.

P57

Morphological and Molecular Characterization of Intramedullary Astrocytomas

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Introduction: Intramedullary astrocytomas (IMAs) are very rare tumours accounting for 1% of all central nervous system tumours. Moreover, IMA surgery is still highly challenging. Very few data are available on the pathogenesis, clinical evolution, and molecular profile of IMAs. While molecular alterations are now essential to establish the diagnosis of cerebral tumours, the grading of IMAs is still controversial since no specific classification is available. Recent reports associated *BRAF* molecular alterations and the *H3F3A K27M* mutation with intramedullary Low (LGAs) and High Grade Astrocytomas (HGAs), respectively. Thus, these tumours seem to be morphologically and molecularly different from their brain counterparts. It is not only a diagnostic problem but also a clinical issue regarding the need to develop novel targeted therapies to improve patient outcomes. The purpose of this study is to apply molecular testing to decipher the molecular landscape of IMAs.
Material and Methods: We reported 41 IMAs samples for which we collected clinicopathological and molecular data from 36 patients (1989 to 2017). *Next Generation Sequencing* (NGS) was performed to characterise these tumours using a panel analysed thirty genes involved in glioma pathogenesis. A Fusion Panel that analysed *KIAA1549-BRAF* fusions was also applied on these samples.
Summary of Results: Our series included 32/41 LGAs and 9/41 HGAs. Forty-two percent of LGAs harboured a *KIAA1549-BRAF* fusion. In contrast, 56% of HGAs (5/9) harboured a *H3F3A K27M* mutation in our series. Moreover, other mutations with potential clinical significance were found, such as *KRAS*, *BRAF*, *NF1*, *TP53*, *ATRX* or *PIK3CA*, while only one case in this series was *IDH1* mutated.
Conclusions: As proposed by certain research teams, *H3F3A K27M* status could be a diagnostic and prognostic biomarker. In our study, only HGAs are *H3F3A K27M* mutated. In the same way, *KIAA1459-BRAF* fusion could be a diagnostic biomarker for LGAs.

P58

Radiation-Induced Brain Injury: The Role of Astrocyte Senescence and Δ133p53

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Purpose of study: Despite the benefits of cancer radiotherapy, patients experience side effects for which effective treatments are not available. These side effects are particularly detrimental in patients receiving cranial radiation and can include progressive, life-long and debilitating neurocognitive dysfunction. We investigated the potential role of cellular senescence in promoting radiation-induced brain injury.
Methods: Immunohistochemical staining was performed to identify cells expressing senescence-associated markers in irradiated brain tissue and their prevalence was compared to non-treated cancer patients. Additionally, primary human astrocytes were exposed to radiation in vitro, and expression of Δ133p53, an inhibitory form of p53, was examined along with induction of senescence-associated functions. Lentiviral overexpression of Δ133p53 was performed to investigate its role in regulating radiation-induced cellular senescence and astrocyte-mediated neuroinflammation.
Summary of results: Astrocytes expressed senescence markers p16INK4A and Hp1γ in all irradiated patient tissues and had higher labeling intensity in irradiated tissues (n=7) compared to untreated controls (n=10). Cellular senescence was also induced in human astrocytes irradiated in vitro. These astrocytes have diminished expression of Δ133p53 and adopt a neurotoxic phenotype as demonstrated by increased senescence-associated beta-galactosidase activity, increased expression of p16INK4A, and increased production of IL-6. Mechanistically, Δ133p53 inhibits radiation-induced astrocyte senescence, promotes DNA repair in irradiated astrocytes, and prevents astrocyte-mediated neuroinflammation.
Conclusions: The p53 isoform, Δ133p53, regulates radiation-induced astrocyte senescence and the neurotoxic senescence-associated secretory phenotype (SASP), suggesting a potential target for reducing neuroinflammation and promoting neuron health in radiation-induced brain injury in cancer survivors.

P59

Characterization of the Immune Response in Alzheimer's Disease

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Alzheimer's Disease (AD) is the most common neurodegenerative disease. Increasing life expectancy ensures that its prevalence will rise even further, posing an indisputable burden in public health worldwide. The enigmatic pathogenesis currently hampers any attempt to identify efficient biomarkers that would contribute to early disease detection along with a therapeutic approach that could halt the disease progression. Alongside the most widely accepted hypothesis that implicates the accumulation of senile plaques and neurofibrillary tangles, a robust microglial-mediated neuroinflammatory response is known to characterize AD. However, the role of microglia and inflammation in different stages of AD is not yet determined. In a previous study we found elevated serum levels of the pregnancy zone protein (PZP) to be predictive for the onset of AD. Subsequent localization of this protein in AD brains pointed to its expression in microglia associated with senile plaques. In the present work, we aim to define the involvement of neuroinflammation in different stages of AD. To that aim, we used post-mortem human brain tissue samples of patients with early and advanced AD and age-matched controls for comparison. We performed gene expression profiling using the nCounter ® neuroinflammation panel of NanoString technology. The technique enables the comprehensive multiplex gene expression analysis of 770 genes and the identification of 23 neuroinflammatory pathways. We used RT-PCR and immunohistochemistry to validate our findings. The characteristics of the immune response in the context of AD will be presented and discussed.

P60

The Role of T Lymphocytes in Facilitating Brain Metastasis

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Purpose of the study: Brain metastases are the most common tumours of the central nervous system (CNS). Incident rates vary according to primary tumour origin, whereas the majority of the cerebral metastases arise from primary tumours in the lung (40-50%), breast (15-30%) and melanoma's (5-20%). In a previous study, we discovered that T lymphocytes facilitate brain metastasis of breast cancer. The aim of this study is to scrutinize the influence of T lymphocytes in the formation of brain metastasis for primary lung cancers and melanomas.
Methods: We co-cultured T lymphocytes, isolated from healthy donors, with lung cancer and melanoma cells. Prostate cancer cells were used as a negative control. The influence of T lymphocytes on the ability of the various cancerous cells to form brain metastasis was checked using an in vitro BBB model. In addition, we measured the protein expression profile of the different types of cells with and without T lymphocyte co-culture, using the nano-liquid chromatography-Orbitrap mass spectrometry (nano-LC/MS). We further validated our significant findings by RT-PCR.
Summary of results: T lymphocytes increased the ability of the various types of cancer cells to cross the in vitro BBB model. Importantly, T lymphocytes increased the ability of cancer cells known to metastasize to the brain to a much larger extent, when comparing with prostate cancer cells. The proteome measurements enabled identifying differentially expressed proteins related to the T cell response. In addition, some proteins associated with migration were overexpressed after co-culturing with T lymphocytes. Currently, the functional relevance of the identified proteins/genes is under investigation.
Conclusions: T lymphocytes increase the ability of cancerous cells to cross the BBB. This finding highlights the importance of increasing the efficiency of the immune system in the primary tumours to prevent cancerous cells from escaping.

P61

This abstract has been withdrawn

P62

Relationship Between *KRAS* Activation and Histological Phenotype in Gastric Cancer

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Purpose of study: In lung, pancreatic and ovarian cancer *KRAS* mutation (*KRAS*mut) characterises a mucinous, ductal and serous histolological phenotype, respectively. *KRAS*mut or *KRAS* amplification (*KRAS*amp) likely results in *KRAS* activation (*KRAS*act). In gastric cancer (GC) the reported frequency of *KRAS*mut and *KRAS*amp varies between 0-29% and 1-9%, respectively. The aim of this study was to investigate whether *KRAS*act in GC is related to histological phenotype using the Lauren and the Japanese Gastric Cancer Association (JGCA) classification.
Methods: Digitized Haematoxylin/Eosin stained slides from 538 GC resection specimens (Japanese series (KCCH): 243 (45.2%), The Cancer Genome Atlas (TCGA): 295 (54.8%)) were classified according to JGCA and Lauren by at least two independent observers. The relationship between *KRAS*act status and predominant histological phenotype was investigated.
Summary of results: The most frequent phenotype was tubular2 (tub2) [n=192, 36%], followed by poorly differentiated non-solid type2 (por2) [n=134, 25%], poorly differentiated solid type1 (por1) [n=95, 18%], tubular1 (tub1) [n=41, 8], mucinous (muc) [n=30, 6%], papillary (pap) [n=22, 4%] and signet-ring cell (sig) [n=22, 4%]. *KRAS*act was found in 100 GC (22%), KCCH: n=56, 24%; TCGA n= 44, 19%. Most *KRAS*act GCs were tub2 (n=43, 43%), followed by por1 (n=18, 18%), por2 (n=17, 17%), sig (n=7, 7%), tub1 (n=5, 5%), pap (n=5, 5%), muc (n=4, 4%), not classifiable (n=1, 1%). According to Lauren's classification 60 (60%) *KRAS*act GCs were intestinal-type, 36 (36%) diffuse-type and 4 (4%) mixed. There was no relationship between *KRAS*act and histological phenotype/clinicopathological variables.
Conclusions: In contrast to lung, pancreatic and ovarian cancer, *KRAS*act in GC is not associated with histological phenotype. As *KRAS*act was present in all different JGCA histological phenotypes, we hypothesise that *KRAS*act is a late event in GC occurring after the histological phenotype is established.

P63

Largest Negative Lymph Node Diameter: A Novel Predictor of Good Prognosis in Patients with Oesophageal Cancer

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Introduction: Lymph node (LN) status e.g. number of positive LNs is a key prognostic factor in patients with oesophageal cancer (OeC). Additionally, a high total number of LNs in the oesophagectomy specimen has been related to a better prognosis. Current studies suggests that the immune system plays an important role in cancer control. 'Activated' LNs with follicular hyperplasia are usually larger in size. We hypothesised that the size of the negative tumour draining LNs is related to OeC patient prognosis.
Material and Methods: HE stained sections of LNs from the resected specimen from 307 OeC patients (159 treated with chemotherapy followed by surgery (CS group), 148 treated by surgery alone (S group)) were digitized. The largest diameter of the negative LNs was measured as a surrogate of LN size. The relationship between negative LN diameter and patient overall survival (OS) was explored.
Results: 1041 negative LNs of CS patients and 1017 negative LNs of S patients were analyzed. There was no difference in the largest negative LN diameter between treatment arms (7.53mm and 7.56mm, respectively). Patients were stratified by median LN diameter for survival analysis. S patients with large negative LNs survived significantly longer than S patients with smaller negative LNs (p=0.009). There was no relationship between LN diameter and OS in the CS patients. Subgroup analysis of patients with large LNs showed no OS difference between S and CS patients.
Discussion: This is the first study to suggest that the negative LN diameter has prognostic value in OeC patients. The survival of patients with large LNs is similar in both treatment arms suggesting that this particular patient subgroup does not benefit from pre-operative chemotherapy. We hypothesise that large negative LN size is related to an 'activated' immune system in the tumour draining LNs. Further studies are warranted to confirm our finding in a second independent series and to characterise the negative LNs in more detail.

P64

Sirtuin 1 Polymorphisms, Energy Balance-related Factors, and Risk of Colorectal Cancer by Microsatellite Instability and CpG Island Methylator Phenotype Status

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Background: SIRT1, a histone deacetylase, is involved in maintenance of genetic stability, inflammation, immune response, metabolism (energy-sensing molecule) and colorectal tumourigenesis. We investigated *SIRT1* polymorphisms in relation to colorectal cancers (CRC) characterized by microsatellite instability (MSI) and the CpG island methylator phenotype (CIMP). Considering SIRT1's role in metabolism, we also explored gene-environment interactions for *SIRT1*-related modification of associations between body size, physical activity and early life energy restriction and CRC by MSI and CIMP status.
Methods: The Netherlands Cohort study (NLCS) was initiated in 1986 and includes 120,852 participants in a case-cohort design. Excluding the first 2.3 years of follow-up, excluding subcohort members and CRC cases with no toenail DNA available for genotyping of two *SIRT1* tagging variants (rs10997870 and rs12778366), and excluding CRC cases with no tumour DNA available left 3719 subcohort members and 557 CRC cases (follow-up period 1989-1993). Cox regression was utilised to estimate hazard ratios (HRs) for MSI and CIMP positive and negative tumours by *SIRT1* genotypes.
Results: The rs12778366 polymorphism was inversely associated with MSI CRC (HR=0.41, 95% confidence interval: 0.20,0.88) but not CIMP subtypes. An exploration of gene-environment interactions found no multiplicative interactions using the Wald test, but it uncovered two significant relative excess risks due to interaction (additive interactions) in relation to microsatellite stable CRC.
Conclusions: The results suggest a role for *SIRT1* polymorphisms in colorectal tumourigenesis and a possible role in risk modification of known CRC risk factors.

P65

Towards High-Throughput Lymphocyte Quantification in Haematoxylin Eosin Stained Biopsy Sections by Establishing an Image Analysis Pipeline

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Introduction: Previous studies showed that the extent of lymphocytic infiltration within the tumour (TIL) is a prognostic factor for cancer patients and predicts response to chemotherapy. However, manual TIL estimation is time consuming. In order to investigate the prognostic/predictive value of TIL in diagnostic biopsies of phase III trials of oesophagogastric cancer (OGca) patients, the aim of this project was to establish an automated, high-throughput procedure to detect lymphocytes in HE stained tissue sections.
Methods: Nine slides with endoscopic biopsies from OGca patients with different HE staining intensities were digitised and sub-sampled to allow rapid analyses using MIM software (HeteroGenius, UK). In an iterative process, slides were manually annotated to train the software to find tissue pieces and differentiate lymphocyte nuclei from other nuclei. Visual assessment followed and the process was repeated until the observer was satisfied.
Results: A colour model was built to outline the irregularly shaped endoscopic biopsy tissue pieces allowing the measurement of lymphocytes/biopsy. Another colour model was built to recognise lymphocyte nuclei within the tissue section using watershed separation/other software tools to segment individual nuclei for counting. The homogeneity of shape, colour and size of lymphocytic nuclei, made this efficient. Nevertheless, satisfactory nuclei detection required repeated manual annotation of a total of 2590 nuclei spread over 12 different subsamples and extensive visual quality control.
Discussion: We established a pipeline for high-throughput counting of lymphocyte nuclei in digitized HE stained slides of endoscopic biopsies from OGca patients. This pipeline will not provide information on lymphocyte subtypes, but gives quantitative information on overall TILs/area, a prognostic and predictive factor in breast cancer. Further development is required to differentiate non-lymphocytic cells including tumour cells.

P66

Lymph Node Response to Chemoradiotherapy in Oesophageal Cancer Patients: Relationship with Current Guidelines for Elective Lymph Node Irradiation

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Background: Current treatment for oesophageal cancer (OeC) is neo-adjuvant chemoradiotherapy (nCRT) or chemotherapy followed by surgery. As recently shown presence of lymph node metastasis (LNmets) and tumour regression grade (TRG) in LNmets are predictors for overall survival (OS). The aim of this study was to investigate if TRG of LNmets is related to their location within the radiation (RT) field.
Methods: TRG in resected LNs of 120 OeC patients treated with nCRT and surgery was retrospectively analysed and classified as: (A) true negative, no tumour regression; (B) with tumour, no regression; (C) with tumour and regression; (D) complete regression of tumour. Anatomical location of all LNs was marked in the resected specimen by the surgeon. LN regions were identified on radiotherapy planning-CT scans to determine if LNs were located within or outside the planned target dose. Relationship with survival was analysed.
Results: In 65 (54%) patients, 275 LNs contained tumour, the majority with signs of TRG: 61 B, 111 C and 103 D. 72% LNs were located within the RT field, only 13 nodes (5%) in 9 patients received <10% of the planned RT-dose. These LNs were mainly located in the paratracheal/subcarinal LN region, while the primary tumour was in the distal oesophagus or gastroesophageal junction. One LN showed complete regression while located outside the RT-field. Univariate analysis showed that presence of LNmets with no TRG in the RT-field is correlated with poor OS.
Conclusion: This is the first study to suggest that TRG of individual LNs is independent of RT-fields and can be heterogeneous within one patient or the same LN region. Given the low incidence of LNmets outside the RT-field, mainly in remote regions, current guidelines for elective LN irradiation appear to be appropriate with current imaging techniques. However, further research is needed to detect LNmets more accurately during clinical staging and to better understand the heterogeneous TRG seen in OeC patients.

P67

Association of Chromosomal Instability, Microsatellite Instability and CPG Island Methylator Phenotype with Postcolonoscopy Colorectal Cancer in a Population Based Study

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Background and aim: Over 50% of the postcolonoscopy colorectal cancers (PCCRCs) (i.e. CRC diagnosed after a colonoscopy that excluded cancer) originate from missed precursor lesions. The biologic pathway of these lesions is unclear. We hypothesized that PCCRCs originate from subtle appearing non-polypoid (NP) adenomas and sessile serrated lesions (SSLs). In a population based study, we examined the occurrence of chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) in PCCRCs and prevalent CRCs.
Methods: We identified all PCCRCs diagnosed from 2001 to 2010 in a large gastroenterology practice from the Netherlands. PCCRCs were defined as cancers occurring within 5 years after a complete index colonoscopy. Only missed and newly developed PCCRC were included. Whole genome DNA copy number changes and mutation status of genes commonly affected in CRC (APC, KRAS, BRAF, FBXW7, PIK3CA, NRAS, SMAD4 and TP53) were examined by shallow whole-genome sequencing and targeted sequencing, respectively, using Illumina next generation sequencing platforms. MSI and CIMP status were examined using the pentaplex marker panel from Promega and the Weisenberger CIMP panel using methylation-specific PCR, respectively. Results were adjusted for age and gender.
Results: In total, 120 PCCRCs and 100 prevalent CRCs were examined. Regarding DNA copy number alterations, PCCRCs contain less often 18q ($p<0.001$) deletions than prevalent CRCs. Furthermore, PCCRCs contain less frequently APC ($p=0.04$), NRAS ($p=0.03$), and TP53 mutations ($p=0.02$) than prevalent CRCs. In contrast, MSI ($p=0.01$), CIMP ($p=0.03$) and BRAF mutations ($p=0.05$) were more frequent in PCCRCs than in prevalent CRCs.
Conclusion: Both CIN and MSI pathways are associated with the occurrence of PCCRC. The molecular profiles support the hypothesis that NP adenomas and SSLs are at the origin of PCCRCs.

P68

The Long Non-Coding RNA CYTOR Drives Colorectal Cancer Progression by Interacting with NCL and Sam68

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Long non-coding RNAs (lncRNAs) function as key molecules in cancer progression. The lncRNA CYTOR plays oncogenic roles in multiple types of cancer, yet the detailed molecular mechanisms of those roles remain unknown. Here we reported that the lncRNA CYTOR was significantly up-regulated in CRC samples and associated with poor prognosis, promoting proliferation and metastasis in vitro and in vivo. NCL and Sam68 could recognize their specific motifs and directly bind to EXON1 of CYTOR. Moreover, EXON1 was the key functional site mediating the interaction of CYTOR with NCL and Sam68. NCL and Sam68 functioned as oncogenes to promote CRC progression. Furthermore, we confirmed that the heterotrimeric complex of CYTOR, NCL and Sam68 activated the NF-κB pathway and EMT to contribute to CRC progression. In Conclusion, CYTOR plays important roles in CRC progression by interacting with NCL and Sam68 and may serve as a prognostic biomarker and/or an effective target for CRC therapies.

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N_LyST: A Simple and Rapid Screening Test for Lynch Syndrome

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Aims: We sought to use PCR followed by high-resolution melting analysis to develop a single closed-tube screening panel to screen for Lynch syndrome. This comprises tests for microsatellite instability (MSI), MLH1 methylation promoter and BRAF mutation.
Methods: For MSI testing, five mononucleotide markers (BAT25, BAT26, BCAT25, MYB, EWSR1) were developed. In addition, primers were designed to interrogate Region C of the MLH1 promoter for methylation (using bisulphite-modified DNA) and to test for mutations in codon 600 of BRAF. Two separate cohorts from two different institutions, A (n=99, 46 with MSI, 53 being microsatellite stable (MSS)) and B (n=88, 45 MSI, 43 MSS) were tested.
Results: All the cases (n=187) were blind tested for MSI and all were correctly characterised by our panel. The MLH1 promoter and BRAF were tested only in the first cohort. Successful blinded analysis was performed on the MLH1 promoter in 97 cases. All MSS cases showed a pattern of non-methylation while 41/44 cases with MSI showed full methylation. The three cases with MSI and a non-methylated pattern had aberrations in MSH2 and MSH6 expression. BRAF mutation was detected in 61% of MSI cases and 11% of MSS cases.Finally, 12 cases were blind screened by using the whole panel as a single test. Of these, five were identified as MSS, four as MSI/non-LS and three as MSI/possible LS. These results were concordant with the previous data.
Conclusion: We describe the N_LyST. This is a quick, simple and cheap method for screening for Lynch syndrome.

P70

Three-Dimensional Co-Culture of Colorectal Cancer Spheroid with Cancer-Associated Fibroblast as a Model to Study Immune Cell Modulation

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Aims: Colorectal cancer (CRC) is a complex disease in which inflammation is often a prominent feature. Hence, it serves as an appropriate paradigm to unveil the clinical importance of tumour microenvironment especially the infiltrating immune and inflammatory cells in the pathology of the disease. A 3-dimensional (3D) in vitro spheroid co-culture model that allows the interaction with stromal components is considered an essential element in developing a more clinically relevant tumour model. In this project, we aim to establish a model consisting of different subtypes of CRC and cancer-associated fibroblast (CAF), a major component of the tumour microenvironment, to study the role of immune cells, for example, macrophages, in CRC biology.
Material and Method: CRC cell lines, HCT116 and DLD-1, and cancer-associated fibroblast (CAF) isolated from CRC patient tumour tissue were seeded together at the total number 2000 cells/well on 96-well round bottom ultra-low cluster/attachment plates at different ratios to form tumour spheroids. Then, different numbers of monocytes isolated from a healthy human volunteer were added into the CRC-CAF co-culture system. The CRC cells and CAF were stably transduced with TdTomato and GFP fluorescent proteins respectively for microscopic visualisation and monitoring of growth. Spheroid growth was also monitored by volumetric measurement using image analysis software (Fiji).
Results and Discussion: Co-culture of HCT116 showed that the presence of CAF increased CRC spheroid growth compared to mono-culture of the cancer cells. Meanwhile, no growth stimulation of CAF-enriched culture of DLD-1 cells was observed despite the smaller size of the spheroids. Similarly, no enhancement of HCT116 growth was observed when monocytes were added to the co-cultures yet this resulted in the more disrupted morphology of spheroids.
Conclusion: This preliminary study highlights the differential tumour growth enhancement by CAF stimuli on different types of CRC.

P71

Testing for PIK3CA and BRAF Mutations in Colorectal Cancer with Microsatellite Instability

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Background and Objectives: Colorectal Cancer (CRC) is the third most prevalent type of cancer and a leading cause of mortality worldwide. Microsatellite Instability (MSI) occurs in 15% of all CRCs, forming a phenotypically and molecularly unique group of tumours. BRAF has repeatedly been reported to be mutated in sporadic CRCs with MSI. A significant association between mutations of KRAS and PIK3CA have been previously shown to exist in CRC. Since both BRAF and KRAS form part of the same (MAPK) signalling pathway, this study aimed to explore the distribution of PIK3CA and BRAF mutations and the association between them in the sporadic MSI subgroup of CRC.
Methods: Genomic DNA extracted from 49 Formalin Fixed Paraffin Embedded (FFPE) CRC cases with MSI were used. Nested Polymerase Chain Reaction (PCR) followed by High-Resolution Melting Analysis (HRMA) were utilised in the detection of somatic mutations in hotspots of PIK3CA (exon 9 and 20) and BRAF (exon 15) oncogenes. The statistical analysis was carried out by a Pearson chi-squared test.
Results: The mutation rate was 21.7% in PIK3CA (13% in exon 9 and 8.7% in exon 20) and 58.7% in BRAF (exon 15). None of the 5 MSI MLH1 proficient tumours harboured BRAF mutations. Concomitant mutations between PIK3CA and BRAF occurred in 3 (6.5%) cases. There was no statistically significant association between PIK3CA and BRAF (p=0.067).
Conclusion: Although both KRAS and BRAF form part of the same MAPK signalling pathway and are both mutated in CRC, their role in colorectal tumour biology may be distinct. BRAF has a major influential role in the development of sporadic MLH1 deficient MSI tumours. Hence, PIK3CA may have a role in a minor subset of MSI tumours which are discrete to BRAF.

P72

Intra-Tumour Ki67 Expression of Colorectal Cancer Does Not Associate with the Tumour Genetic Features

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Aims: Colorectal cancer (CRC) with microsatellite instability has been widely reported to have better prognosis than microsatellite stable (MSS) CRC. Meanwhile, Ki67 is a proliferative marker that commonly used in clinical pathology practice to estimate the growth fraction of the tumour. However, the prognostic role of Ki67 expression in CRC is contradictory and differential results have been reported on various cohorts of patients. This project aimed to correlate the expression of Ki67 with microsatellite instability and mutation status on CRC using a digital pathology platform.
Materials and Methods: For this study, 99 formalin-fixed, paraffin-embedded (FFPE) tumour blocks of resected primary CRC were retrieved from the histopathology department of Nottingham University Hospital, UK. Immunohistochemistry (IHC) for Ki67 was performed on freshly cut 4µm-thick tissue sections using automated slide staining system (Benchmark ULTRA, Ventana Medical System, Tucson, AZ). Digital image analysis (DIA) for Ki67 expression within the tumour area was carried out on whole slide image (WSI) using a hexagonal tiling (HexT) approach developed on HALO software (IndicaLabs, Corrales, NM). Furthermore, mutation of the hotspot regions of KRAS, BRAF, and PIK3CA genes was screened by fluorescence-based PCR followed by high resolution melting analysis (HRMA) on the DNA extracted from the FFPE sections.
Results and Discussion: Tumours with MSI showed a similar level of Ki67 expression to those with MSS. Moreover, no significant association was found between Ki67 and somatic mutations of common hotspots.
Conclusion: The results suggest that MSI status and common genetic mutations cannot predict tumour proliferation.

P73

The Role of CD26 in Promoting the Metastatic Phenotype in Colorectal Cancer: An *In Vitro* Study

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Purpose of the study: CD26, dipeptidyl peptidase IV, a putative stem cell marker, has been linked to poor clinical outcome and distant metastases in colorectal cancer [PLoS One. 2014; 9(5)] but its functional mechanisms are not fully elucidated. This study aimed to investigate the possible functional mechanisms of CD26 in generating the metastatic phenotype in colorectal cancer (CRC).
Methods: CD26 expression was determined *in vitro* in four CRC cell lines (HCT116, SW480, SW620 and DLD1) by Western blots. Functional changes in proliferation, migration, invasion and wound healing were assessed after both transient and stable CD26 knockdown. Effects on epithelial mesenchymal transition (EMT), angio/lymphangiogenesis and the TGF-beta pathway were also investigated.
Results: HCT116 cell line expresses the highest amount of CD26 protein and shows more migratory abilities than SW480, a lower expresser. SW620, in contrast, expresses negligible CD26. Both transient and stable CD26 knockdown produced a significant reduction in migration and invasion of CRC cells but not proliferation. Wound healing was also significantly abrogated by CD26 knockdown. CD26 loss reverses the Cadherin switch, reduces MMP7 and Snail expression and lowers angiogenic (VEGF B) and lymphangiogenic (VEGF C) growth factor production with concomitant loss of TGFβ expression. Within the TGFβ pathway, there was no effect on SMAD4 but reduction in ROCK1 after CD26 knockdown.
Conclusion: CD26 plays a vital role in CRC migration and invasion independent of proliferation. Multiple molecular events such as EMT, angiogenesis and growth factor production may be affected by CD26 indicating its manifold roles in generating a pro-metastatic phenotype.
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P74

Investigating the Role of PSMD Proteins (6, 10) in Colorectal Cancer

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Purpose of the study: Molecular mechanisms of vascular invasion (VI) contributing to cancer metastates are not well delineated. METABRIC breast cancer series data suggests that the PSMD family, members of the ubiquitin-proteasome system, may serve a transcriptomic hub promoting lymphovascular invasion. This study investigated the role of the PSMD proteins (6 & 10) in colorectal cancer (CRC) for functional effects and clinico-pathological significance.
Methods: siRNA knockdowns of PSMDs (6 & 10) were used to assess effects on proliferation, migration, and invasion in the matched pair of CRC cells: SW480 (primary) and SW620 (metastatic). Protein expression was examined by immunohistochemistry on CRC tissue microarrays (n=85).
Results: PSMD 6 and 10 knockdown had no effect on proliferation in SW620 but reduced proliferation in the SW480 cell line. Invasion and migration assays with SW620s showed that both PSMD 6 and 10 knockdown significantly reduced invasive and migratory potential compared to controls. Preliminary results however showed only a marginal decrease in VEGF-A and VEGF-C protein levels in both protein knockdowns. On immunohistochemistry, no significant associations with grade, T/Dukes stage or lymph node involvement was seen except for a borderline negative correlation (p=0.049) with VI and nuclear PSMD expression.
Conclusions: Results suggest that PSMDs 6 and 10 may contribute to the migratory phenotype in metastatic CRC while promoting proliferation in the primary. The negative correlation with VI for the nuclear protein may be due to activated DNA damage response, a known function of nuclear PSMD proteins. Immunohistochemistry on larger series of CRCs will help confirm whether the effect of PSMD proteins on VI is likely to be a passenger effect within a complex molecular network.
Supported by the Pathological Society of Great Britain and Ireland, Academy of Medical Sciences and the Nottingham Molecular Pathology Node

P75

An Audit of the Specimen Quality and Reporting of Medical Liver Biopsies

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Background: Medical liver biopsies are undertaken when information required for patient management cannot be obtained by non-invasive means. The benefit should justify the risk of biopsy. Tissue Pathways for medical liver biopsies gives guidelines on the recommended items to be included in the report, emphasising the importance of clinical relevance. Discussion with clinician should be recorded on the report.
Aim and objectives: To determine the percentage of medical liver biopsy reports that meet the recommended criteria for report content (section 8, Tissue Pathways for liver biopsies for the investigation of medical disease and for focal lesions, 2014).
Methods: Tru cut biopsies of liver were retrieved from the LIMS for the years 2016 and 2017. Any biopsies undertaken for non-medical causes (malignancy or cysts) were excluded. Rcpath minimum criteria was used to assess compliance.
Results: There were a total of 142 medical liver biopsies in the year 2016 and 2017. Clinical information was considered sufficient in 61.3% of the reports. Length of biopsy was reported in all cases. Number of portal tracts was mentioned in only 37.3% reports. Biopsy was considered adequate in 82.4% cases. Clear histological description, disease stage, clinical comment and a concise summary was present in more than 90% of cases. In less than 10% of cases there was any mention of intradepartmental consultation, discussion with clinician or referral to an outside facility. All the reports addressed the clinical indication. Thirty-six percent of reports were issued within 7 days of specimen reception.
Conclusion: There is a wide communication gap between pathologist and clinician. The turn around time for most reports is more than what is recommended by RCpath guidelines.
Recommendations for improvement: Every case should be discussed with the clinician. Portal tracts should be added to every report and sign out time should be improved.

P76

$p16^{INK4a}$ and HPV E4 Expression Patterns in Intraepithelial Neoplasia Caused by Low-Risk and High-Risk HPV Infection: A Study by Laser Capture Microdissection

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Purpose of the study: Immunohistochemical (IHC) biomarkers offer the possibility of standardised, reproducible grading of anal intraepithelial neoplasia (AIN) based on progression of HPV infection from transient productive infection to complete neoplastic transformation. We investigated the expression patterns of IHC biomarkers p16 (transformation), and HPV E4 (productive infection), in low-grade and high-grade AIN caused by low-risk (lr) and high-risk (hr)HPV infections.
Methods: A reference grading of AIN was established using expert, consensus, subjective HE diagnoses for the worst lesion present in 116 anal biopsies from HIV+ MSM in Amsterdam: normal squamous epithelium 17, AIN1 36, AIN2 39, AIN3 24. The causative genotype of the worst lesion on biopsy was identified using laser capture microdissection (LCM) SPF10-PCR.
Summary of results: In hrHPV lesions (n=53), p16 staining positively correlated with lesion severity, showing diffuse p16 staining above the lower third of the epithelium in almost all AIN2-3 (37/38, 97%). Of lesions caused by lrHPV (57), most showed an extensive patchy staining pattern (46/57, 81%), including AIN2+ lesions (18/23, 78%). Overall, 1/17 (6%) of the normal biopsies and 3/24 (13%) of AIN3 were HPV E4 positive, and were caused by hrHPV. E4 positivity was found in 15/36 (42%) of all AIN1 and 17/39 (44%) of all AIN2, with no significant differences found between lesions caused by lrHPV and hrHPV.
Conclusions: Our results show that lrHPV and hrHPV infections can both cause high-grade AIN in HIV+ MSM but show different IHC expression patterns. Therefore, it is important to know the causative HPV genotype of a lesion. Studies that further explore the use of p16 and E4 in practice to distinguish transforming lesions at high risk of progression from productive lesions, are necessary.

P77

The Intraepithelial Lymphocyte Count in Duodenal Biopsies: Does CD3 Immunohistochemistry Give Comparable Results to Haematoxylin and Eosin Staining?

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Purpose of the study: Intraepithelial lymphocyte (IEL) count is assessed in duodenal biopsies as an important diagnostic feature of conditions such as coeliac disease. Immunohistochemistry for the T cell marker CD3 has been recommended as an adjunctive method, but it is not clear whether the counts obtained from haematoxylin and eosin (H&E) stained slides are comparable with counts from CD3 immunohistochemistry. The aim of this study was to compare IEL counts from H&E and CD3 and examine the effect on inter-observer variability.
Methods: The number of IELs per 100 enterocytes was counted in 35 paired H&E and CD3 sections by six pathologists. The counts were categorised into three groups: normal (<25 lymphocytes), raised (25-40 lymphocytes) and very high (>40 lymphocytes).
Summary of results: For H&E stained sections the mean IEL count was 35.2 and for CD3 the mean count was 49.7. This difference was significant ($p = 0.007$). The pooled H&E vs CD3 counts from all pathologists (total 210 counts in each group) were as follows: normal 62 vs 38; raised 67 vs 52; very high 81 vs 120. The difference in diagnostic categorisation using the two methods was significant ($p < 0.001$). Fleiss kappa analysis demonstrated moderate agreement between observers for both assessment of H&E (kappa = 0.63, 95% confidence interval 0.55 to 0.72) and CD3 immunostaining (kappa = 0.72, 95% confidence interval 0.63 to 0.80).
Conclusions: CD3 immunohistochemistry was associated with significantly higher IEL counts than assessment on H&E but did not significantly reduce inter-observer variability. There were differences in diagnostic categorisation using the two methods. IEL counts derived from CD3 and H&E may not be comparable to each other.

P78

miRNA Expression Profile of Gastric Fundic Gland-Type Neoplasm

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Purpose: Gastric fundic gland-type neoplasm (FGTN) is a recently recognized rare variant of gastric epithelial neoplasia that mainly arises from non-atrophic gastric mucosa without *H. pylori* infection. Although some genetic alterations have been reported, the molecular basis of FGTN remains unclear. Here we investigated expression profiles of immunohistochemistry (IHC) markers, including MUC2, MUC5AC, MUC6 etc. and microRNA (miRNA) in FGTN to clarify the difference between FGTN and other conventional well-differentiated (w/d) adenocarcinomas.
Methods: For miRNA analysis, RNA was extracted from microdissected formalin-fixed paraffin-embedded tissue. Comparative analysis of 16 FGTNs and 16 conventional w/d adenocarcinomas was conducted using IHC, miRNA microarray, real-time polymerase chain reaction (PCR), and miRNA in situ hybridization.
Results: FGTN and conventional w/d gastric adenocarcinomas were stratified into clusters based on hierarchical clustering of the IHC markers and miRNA expression. The cluster patterns were unique to each histologic type. The miRNA expression profile revealed different miRNA signatures between FGTN and conventional w/d gastric adenocarcinoma, especially in miRNAs related to cancer progression. The miRNA expression was confirmed by real-time PCR and miRNA in situ hybridization.
Conclusions: Expression profiles of IHC markers and miRNA indicated that FGTN and conventional w/d gastric adenocarcinoma are separate entities.

P79

Primary Anorectal Amelanotic Melanoma: A Review of Two Cases

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Background: Anorectal melanoma is a rare malignancy comprising 0.3% of melanomas and 0.8% of anorectal malignancies. The median age at diagnosis is 66 years with a female predominance. Thirty percent are amelanotic and can be confused with benign conditions like hemorrhoids.
Methods: We report two cases of anorectal amelanotic melanoma presenting at a very young age and with no previous history of melanoma in any part of the body. The tumour measured 2x2x2cm. Both had surgical excision of the lesion. Immunohistochemistry was done using CK, S-100, Desmin, HMB- 45.
Results: The patients were both female aged 27 and 32 years respectively. Rectal bleeding was the commonest symptom in both cases. Duration of symptoms was three months. Microscopy showed both tumour were located in the submucosa and composed of markedly pleomorphic cells with hyperchromatic nuclear, prominent eosinophilic nucleoli with granular cytoplasm. No melanin pigment was seen. Immunohistochemistry showed positive stain for S-100, HMB-45 and negative for CK and Desmin.
Conclusion: Anorectal amelanotic melanoma displays the same immunohistochemistry with its cutaneous counterpart however, the age at presentation differs with almost all cases of anorectal amelanotic melanoma reported in the literature. We advocate further molecular testing to identify possible reason/risk factors our patients presented at a younger age and also identify possible pathways for novel therapy in the future.

P80

Androgenetic/Biparental Mosaicism with Molar Pathology: Case Report

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Purpose of the study: Hydatidiform mole (HM) is a proliferative disorder of villous trophoblast characterized by hydropic chorionic villi and trophoblastic hyperplasia. Subclassification of HM into complete or partial mole is important to assess the risk of developing persistent gestational trophoblastic disease (GTD). Rare cases of androgenetic/biparental mosaicism with overlapping morphological features of HM exist which are regarded as having a risk of persistent GTD, as yet unquantified. Here we present a case of androgenetic/biparental mosaicism.
Methods: This case was sent to the HM service for review by a specialist pathologist. The submitting pathologist suspected molar pregnancy. Pathological analysis, p57 immunohistochemistry (IHC) and molecular genotyping of laser microdissected tissue were performed.
Summary of results: Sections showed enlarged hydropic villi with predominantly lobulated outlines and widespread cistern formation. Scattered trophoblast pseudoinclusions were seen but there was no evidence of non-polar trophoblast hyperplasia. IHC for p57 displayed an aberrant pattern being entirely negative in villous stromal cells but positive in villous cytotrophoblast. Laser capture microdissection and genotyping on stromal cells showed homozygosity at all alleles, with no evidence of inheritance of any maternal alleles. Analysis of cytotrophoblast cells revealed a heterozygous diploid complement consistent with biparental inheritance. In view of the presence of paternal isodisomy, the patient had the standard follow-up for a complete HM.
Conclusions: Taken together, these findings indicate presence of two cell lines consistent with androgenetic/biparental mosaicism. These studies highlight the importance of a multidisciplinary approach in classification of equivocal cases. An international study is currently underway to elucidate the clinical risk associated with such cases where the presence of an androgenetic component is evident.

P81

A Case of Malignant Uterine PEComa

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Introduction: Perivascular epithelioid cell tumour (PEComa) is a rare subtype of mesenchymal origin tumours, and is composed of perivascular epithelioid cells with specific histologic and immunohistochemical features. As these are a rare entity in the gynaecological tract, we would like to present a case of PEComa of the uterus.
Case Presentation: A 49 year-old female presented with large pelvic masses within the Pouch of Douglas. She under went a debulking operation with hysterectomy and anterior resection of the colon. An intraoperative frozen section showed these masses to be composed of a high-grade malignancy. Further sections were composed of sheets of polygonal cells with necrosis and abundant mitosis. In addition a spindle cell lesion of the myometrium was noted. Both the lesions were positive for HMB45, S100 and SMA. The appearances and the myo-melanoytic immunoprofile are consistent with a malignant uterine PEComa.
Discussion: PEComa is a poorly defined neoplasm that is characterised by varying amounts of spindle and epithelioid cells that display immunoreactivity for melanocytic and smooth muscle markers. PEComas are tumours of uncertain malignant potential however a mitotic count and necrosis are features of aggressive behaviour. PEComas can also be related to the tuberous sclerosis.

P82

An Audit of the Diagnostic Accuracy of Intraoperative Assessment of Pelvic Masses

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Introduction: Preoperative biopsy diagnosis of pelvic masses is flawed because of sampling error and the potential for cyst rupture and upstaging of ovarian malignancy. Intraoperative frozen section assessment can be an excellent adjunct in the management of pelvic pathology. However, as with all areas of diagnostic pathology, frozen sections have limitations. We undertook an audit to assess the accuracy of frozen section diagnosis of pelvic masses.
Objective: To ensure that the Oncology Centre is achieving high rates of accuracy when reporting frozen sections of pelvic/ovarian masses.
Method: A retrospective audit was conducted looking at intraoperative assessment of pelvic masses between 1st January 2013 and 20th March 2016. For each case the frozen and paraffin diagnoses were divided into four categories: benign, borderline +/- microinvasion, primary malignant and metastasis. The accuracy of assessing the presence or absence of invasive malignancy, along with the sensitivity and specificity for detecting invasive malignancy were established.
Results: One hundred and seventy cases were retrieved. The overall accuracy of intraoperative diagnosis of invasive malignancy was 97%. The overall accuracy of intraoperative designation of the cases into one of the four categories was 92% (157/170). The sensitivity and specificity for the detection of invasive malignancy were 93% and 100% respectively. No false positive diagnosis of invasive carcinoma was made.
Conclusions: The accuracy of frozen section diagnosis for pelvic masses is at the high end of the range expected from the literature. This procedure is a reliable adjunct in the surgical management of pelvic masses.

P83

Ectopic Adrenal Cortical Adenoma: A Case Report

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Ectopic adrenal tissue is rare and can be found in locations such as the celiac axis, the broad ligament, adnexa of testis and the spermatic cord. We report an unusual case of a 57 year old female with an ectopic adrenal cortical adenoma in the right pelvis. She presented with ascites and a raised CA125. CT showed a solid echogenic mass with vascularity in the right pelvis. Cytology of the ascitic fluid did not reveal any malignant cells. The mass was well away from the right kidney and adnexa. Histology confirmed it to be a benign adrenocortical adenoma. The patient had undergone a hysterectomy, right salpingooophorectomy and a left cystectomy fifteen years prior to this.

P84

Seromucinous Borderline Tumours of the Ovary: A Precursor to Clear Cell Carcinoma?

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Purpose of the study: We describe an unusual case of clear cell carcinoma and seromucinous borderline tumour present in the same patient in opposite ovaries on a background of endometriosis. We investigate whether the two tumours are separate entities or should be considered as part of a spectrum of neoplasms derived from endometriosis.
Methods: We employed morphological assessment, immunohistochemistry and targeted next generation sequencing of the two tumours from our case study. We found five cases of seromucinous borderline tumours diagnosed in our hospital in the past 3 years. We compared their demographics, clinical outcome, morphological and immunohistochemical findings.
Summary of results: Gene sequencing of the clear cell carcinoma and seromucinous borderline tumour from our case study showed mutations within the same codons of KRAS and PTEN. Both tumours were WT1 negative and p53 wildtype. The clear cell carcinoma was negative for ER and DPAS while the borderline tumour was positive for both. Review of the five seromucinous tumours in our archives showed that there were focal areas of clear cell morphology in all of them. Five out of six seromucinous borderline tumours showed foci of endometriosis yet only one patient had been diagnosed with the condition.
Conclusions: Clear cell carcinoma and seromucinous tumours are known to be strongly associated with endometriosis but their relationship has not been explored. The genetic sequencing data from our case study suggests that the two tumours originated from the same clone. The morphology of our archived borderline tumours implies that the two types of tumour may be part of a spectrum of endometriosis associated ovarian cancers. The results of this study highlights the need for further characterisation of seromucinous borderline tumours and their relationship to clear cell carcinoma.

P85

A Review of Diagnostic Discrepancies in Gynaecological Pathology Referral Cases at a Tertiary Centre

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Aims: The development of cancer centres and clinical networks has led to an increasing requirement for histopathology review. The Royal College of Pathologists recommends that pathologists audit their patterns and frequency of referrals and record discrepancy rates. The aim of our study was to identify the areas of diagnostic discrepancy in reporting gynaecological pathology referred to a specialised oncology unit at a tertiary centre.
Methods: Gynaecological pathology cases referred for review via the multidisciplinary team were assessed over a 6 month period from March-August 2017. Reports were compared between the referring centre and following specialist review for discrepancies in diagnosis. The implementation of additional immunohistochemistry was also assessed.
Results: A total of 147 gynaecological referral histopathology cases were reviewed (109 biopsies and 38 resections). The final diagnosis was modified in 24 cases (16.3%); 20 of which were biopsies and 4 resections. In 9 cases (6.1%) there was a significant change to the final diagnosis which could conceivably lead to a change in patient management. In 9 cases (6.1%) the review refined the diagnosis by providing a specific subtype of malignancy. In 6 cases (4.1%) there was a minor change to the diagnosis, unlikely to have a significant impact on patient management. Most of the significant diagnostic discrepancies were related to tumour classification. There was a change in grading of malignancies in 15 cases (10.2%). Grading was found to be most discrepant in endometrial endometrioid adenocarcinoma. Additional immunohistochemistry was performed in 26 cases (17.7%).
Conclusions: While there is diagnostic agreement between referring institutions and the specialised cancer centre in the majority of cases, the rate of discrepancies with potential significant impact on patient management is nevertheless considerable and highlights that centralised review of gynaecological histopathology is good practice.

P86

Incidental Finding of a Neurogenic Cyst of the Ovary During Fertility Investigations: A Case Report of a Rare Benign Monodermal Teratoma

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Monodermal teratomas of the ovary other than struma ovarii or carcinoid tumour are incredibly rare. We describe one such cystic tumour in a 39 year old female with the cyst wall composed solely of mature GFAP and S100 positive neuroglial tissue with a prominent vasculature. This case presented during fertility investigations when a 6x7cm cyst was discovered on ultrasound with radiological features of a dermoid cyst. After subsequent spontaneous conception the patient wished not to remove the cyst during the pregnancy and the cyst was only monitored. The true nature of the by now 8cm cyst was discovered during histological examination after removal at Caesarean section at 36+3 weeks. A review of the literature reveals this case to be only the 6th pure case described.

P87

The Role of Reticulin Staining in Sex Cord Stromal Tumours of the Ovary

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Introduction: The diagnosis in sex cord stromal tumours (SCST) is in most cases difficult and often ancillary techniques are necessary. The most common differentiation roots are the granulosa or the theca differentiation. Both have a normal pendant in the ovary – the granulosa cell and the theca cell layer, characterized by a typical reticulin pattern. Sertoli-Leydig cell tumours do not have a physiological counterpart in the ovary. The aim is to investigate the reticulin pattern of adult granulosa cell tumours (aGCT) and thecomas and to define the reticulin pattern of Sertoli-Leydig cell tumours (SLCT).
Methods: This study consists of 113 sex cord stromal tumours between 1991 and 2017. The series consists of 35 aGCTs, 28 thecomas, 12 SLCTs and others. On each case, a reticulin stain was performed on a representative block.
Results: Of the aGCT, 66% showed no reticulin staining, corresponding to the granulosa cell in normal ovary. In the group of thecomas, 68% showed individual cell surrounding, as seen in normal theca layer. In the group of SLCT, 75% showed a staining pattern with nests and cords. In the total group, 68% showed a reticulin pattern that is concordant with the H&E diagnosis. 22% tumours showed a pattern that did not fit with the diagnosis, and 10% showed a staining pattern that was partly fitting with the diagnosis. In the group of tumours with a potential malignant behavior, there was 66 % concordance between H&E diagnosis and the reticulin.
Conclusion: 66 % of aGCT showed a pattern conform normal granulosa cell layer and 68 % of thecomas showed a pattern conform normal theca layer. Most SLCT have a reticulin pattern of nests and cords. In all patients, in 32 % of the cases the staining pattern in the reticulin did not fit with the H&E diagnosis. These discrepancies were equal in the benign and potentially malignant group. Therefore, the reticulin staining pattern can aid in differentiating benign from malignant SCST but must be used with ancillary data.

P88

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

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Phyllodes tumours are uncommon fibroepithelial neoplasms, which usually occur in the breast. They account for 0.3 to 0.5% of all primary breast neoplasms in females. They are subclassified as benign, borderline or malignant, with the majority (50-60%) displaying only benign features. Primary phyllodes tumours of the vulva are exceptionally rare, with only 16 cases previously reported in the literature to date. We present the case of a primary vulvar phyllodes tumour in a 42-year old female. The patient presented with a vulvar mass, which on clinical examination was considered consistent with a vulvar cyst. Macroscopic examination of the specimen revealed a smooth, grey nodule, which was 21mm in maximum dimension. Morphologic examination showed a well-circumscribed biphasic tumour with the classical "leaflike" architecture, comprising fronds lined by secretory epithelium which projected into cystic spaces. There was increased stromal cellularity, but no mitotic activity or cytological atypia was seen. No ectopic breast tissue was identified histologically adjacent to this lesion. Immunohistochemistry showed strong ER positivity in the secretory epithelium with patchy GCDFP15 positivity. The stroma was CD34 positive, and myoepithelial cells underlying the epithelium showed S100 positivity. The findings were consistent with a benign Phyllodes tumour. The histologic origin of vulvar phyllodes tumours is unclear, however the favoured theory is that they derive from the specialised mammary-like glands of the anogenital region. We present a detailed literature review of 17 cases, including our own. This is, to our knowledge, the largest review of primary vulvar phyllodes tumour to date.

P89

Ribosomal Protein S6 Kinase (p70S6K) Expression is Associated with Platinum Resistance and Poor Clinical Outcome in Primary Epithelial Ovarian Cancer

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Purpose of the study: Ovarian cancer is the leading cause of death from gynaecologic malignancy. More than half of ovarian cancer patients are diagnosed at an advanced stage. The mammalian target of rapamycin (mTOR) pathway has a crucial role in the regulation of translation of specific proteins associated with ovarian cancer progression. The major downstream effector of mTOR is ribosomal protein S6 kinase (p70S6K). We aimed to investigate the biological significance of this protein in ovarian cancer.
Methods: Investigation of p70S6K protein expression, was carried out on tissue microarrays of 195 consecutive ovarian epithelial cancers treated at Nottingham University Hospitals (NUH) between 2000 and 2007. Clinicopathological and outcome data were collected.
Summary of results: High cytoplasmic expression p70S6K was associated with higher stage at presentation (p=0.012) and resistance to platinum chemotherapy (p=0.034). Univariate outcome analysis showed an inverse association between P70S6K expression and both progression free survival and Ovarian cancer specific survival (p=0.001) (p=0.048) respectively. Moreover, selecting only the platinum sensitive patients showed inverse association between high expression of P70S6K and local recurrence (p=0.006).
Conclusions: mTORC1 downstream effector p70S6K positive expression predicts platinum sensitivity and local recurrence in ovarian cancer patients. Therefore, this could be a future potential target that could improve patients' survival and reduce tumour recurrence.

P90

A Rare Case of Ovarian Trilinear Carcinoma (Squamous Cell Carcinoma, Endometrioid Adenocarcinoma and Clear Cell Carcinoma) Arising from an Atypical Endometriotic Cyst

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Introduction: Endometriosis is estimated to affect 2-8% of women of reproductive age and is associated with an increased risk of ovarian malignancy. Endometriosis associated ovarian carcinoma is usually of the endometrioid and clear cell subtype, with squamous cell carcinoma rarely reported.
Case Report: An otherwise well 57 year old woman presented with a 2 month history of abdominal pain and distension with normal tumour markers. Pelvic MRI revealed a large complex right ovarian cyst with no regional or distant metastasis. Hysterectomy, bilateral salpingo-oophorectomy, omentectomy and lymph node dissection were performed.
Pathology: The right ovary contained an unilocular cystic mass histologically consisting of a mixed carcinoma composed mainly of G2-3 squamous cell carcinoma (>90%), with G1 endometrioid adenocarcinoma and clear cell carcinoma. The neoplasm was present within an atypical endometriotic cyst. All three carcinomatous components were separate from each other with no intermingling. The peritoneal nodule contained endometrioid adenocarcinoma and the lymph node showed no metastasis. Separate areas of benign endometriosis were also identified. The FIGO stage was IIB.
Discussion: Three theories exist regarding the evolution of ovarian carcinoma within endometriosis: 1) a step-wise progression from atypical endometriosis, 2) inflammatory and hormone microenvironment favouring tumourigenesis and 3) metaplasia within endometriotic glands undergoing malignant transformation. Three discrete tumour components in this case favour each lineage arising separately from atypical endometriosis, rather than heterologous differentiation. Further molecular analysis is desirable to enhance understanding of carcinogenesis.
Conclusion: To the best of our knowledge this is the first case of trilinear carcinomatous transformation of an atypical endometriotic cyst. Recognising the existence of multi-lineage malignant transformation has important clinical implications.

P91

An Audit of Referral and Handling of the Placental Tissue Specimens

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Purpose: The Royal College of Pathologists (RCPath) in consultation with the British Paediatric Pathology Association (BRIPPA), the Royal College of Obstetrics and Gynaecology (RCOG) and the Royal College of Midwives (RCM) introduced guidelines for the responsible handling of placental tissue specimen. Adherence to these guidelines is encouraged as it ensures that minimum standards are met at reduced costs.
Methods: Clinical/obstetric information provided on the histology request form, as well as details of specimen dissection, block selection and histology reports of placental tissue specimen received in our facility from June 2016 to June 2017 were retrieved from the database and reviewed for adherence to the standards proposed by RCPath.
Results: One hundred and fifty seven (157) placental tissue specimens were examined during the period of the audit. The indication for referral, gestational age and birth weight were recorded in the request form in 94.9%, 70.7% and 1.9% of cases respectively. Only 1.9% of cases had all 3 of these minimum data recorded. There was sufficient clinical information for triaging the specimens in 73.9% of cases. Only 26.7% of cases were triaged according to the RCPath guidelines. As a result, all cases were subjected to full examination and histology when 22.3% required no gross or histological examination, 5.7% required only gross examination and only 26.7% required full examination with review of the histology slides. All standard measurements of the specimen were recorded in 83.7% of cases at surgical cut up. However all (100%) were sampled adequately. All cases met the turn-around time of less than 42 days.
Conclusion: Clinical details were poorly recorded in the request forms which precluded proper triaging. Non-adherence to the RCPath guidelines led to over-examination of the tissues. These have cost implications for the rendering of pathologic services.

P92

An Audit of Compliance Against Local Agreed Standards for the Harvesting of Pelvic Lymph Nodes at Dissection

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Aims and Objectives: To ascertain the impact on lymph node yield by sampling all of the remaining fat in pelvic lymph node dissections in order to comply with local recommendations of achieving 15 lymph nodes.
Standards and Criteria: Local recommendations from gynaecological surgeons recommend that a minimum of 15 lymph nodes should be harvested from pelvic nodal dissections.
Baseline Data Sampling: An audit of the laboratory information management system was conducted between January and March 2017, and yielded 1223 gynae specimens. Of these only 15 cases contained pelvic lymph node dissections. 10 out of the 15 cases were bilateral dissections.
Inclusions and Exclusions: Three cases were excluded from the baseline data audit due to incorrect specimen coding.
Personnel: Seven out of fifteen cases were dissected and reported by UHS pathologist (2 different consultants), whilst the remaining 5 cases were dissected and reported by specialty trainees in pathology (4 different trainees).
Summary of Results: Lymph node yields ranged from 1-21 lymph nodes. Out of the 12 cases, 4 had all tissue submitted. These had lower yields of lymph nodes ranging from 1-6, suggesting that there were pre-analytical factors resulting in such yields. The remaining 8 cases ranged from 3-21 lymph nodes, of these cases 62.5% achieved over 15 lymph nodes, and the average nodal yield was ~14 lymph nodes. It is hoped that if all remaining tissue is submitted that we can yield an average of 15 nodes, or achieve higher than 62.5% of cases achieving more than 15 lymph nodes.
Conclusion: Currently the department is not achieving the proposed standard set by local surgeons of 15 lymph nodes. The re-audit in April 2018 will provide further data to assess whether entire processing of these specimens will improve the yield.
Action Plan: Following these results our practice has changed to sampling the entirety of pelvic nodal specimens. Results from the re-audit period in April 2018 will be presented

P93

Mismatch Repair Gene Mutation Detection in Endometrial Cancer: Are Histopathologists Doing Enough?

Ⓟ S Padayachy; E Jaynes

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Lynch syndrome (LS) or hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant syndrome that increases a patient's risk of developing multiple cancers, most commonly colorectal and endometrial carcinoma (EC). This is caused by a germline mutation in one of the DNA mismatch repair genes (MMR); hMLH1, hMSH2, hMSH6, or hPMS2. Current NICE guidelines recommend that all colorectal cancer patients undergo testing for immunohistochemical (IHC) MMR proteins or microsatellite instability (MSI). As such, routine screening of patients with colorectal carcinoma for LS has become increasingly common. A retrospective laboratory information management audit of our department in 2017, demonstrated that out 58 colorectal cancers in patients under the age of 65, 30 had MMR IHC performed (51.7%). However, the lifetime risk of EC is 40-60% which is equivocal or greater than the risk of colorectal cancer, therefore identifying EC patients with LS is believed to reduce their risk of synchronous and metachronous tumours. Routine screening using MMR immunohistochemistry in EC is rarely performed at our centre. Following a local review of hereditary malignancies, an audit of the total number of EC's in patients under the age of 65 diagnosed within 2017 was performed (n=115). Further classification by tumour type was performed: endometrioid (n=102, 88.6%), serous (n=6, 5.2%), clear cell (n=2, 1.7%) and mixed patterns (n=5, 4.3%). Of these cases only 7 had MMR immunohistochemistry performed, due to the recognition of the young patient age by the same histopathologist, one of these patients in retrospect had a family history suggestive of LS. This low number may be due to the lack of awareness of the clinicopathological implications of MMR testing. The diagnosis of endometrial cancer in patients with LS has important clinical implications for both the patient and family members, thus we propose changes to our local practice to address this potential diagnostic oversight.

P94

Analysing the Epidemiology of Molar Pregnancies in England

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Molar pregnancies are a benign form of gestational trophoblastic disease that are relatively rare. However, variances in the incidence of molar pregnancies are observed worldwide with numerous factors proposed to influence this, including: ethnicity, maternal age and changing diagnostic methods. This study aimed to calculate the incidence of molar pregnancies in England, along with an analysis of biological and social factors that may influence the risk of molar pregnancies. We also aimed to examine the methods used for their diagnosis, particularly the contribution of cytogenetics. A retrospective study was performed analysing data for all cases of molar pregnancy in the relevant trusts from 1st January 2012 to 31st July 2017. An overall incidence of 1.62 molar pregnancies per 1000 live births was observed, with an increasing incidence in the White population. Asian ethnicity, extremes of maternal age and primi-gravida status were associated with increased incidence of complete hydatidiform moles, whereas a history of miscarriages was positively associated with partial hydatidiform moles. Histological examination was the predominant method of diagnosis for both types of molar pregnancy, although cytogenetic analysis was also used commonly in the diagnosis of partial hydatidiform moles. Both histological and cytogenetic analysis demonstrated diagnostic limitations with regards to partial hydatidiform moles. Considering our findings, we have proposed recommendations for the accurate and comprehensive diagnosis of future molar pregnancies, specifically a combined genetic and histological approach for the diagnosis of partial hydatidiform moles. Additionally, we have identified areas where further research is needed, particularly the association between nutrition and molar pregnancies and an advanced understanding of the genetic aberrations underlying the disease.

P95

Challenges of Frozen Section in a Rare Liver Mass: A Case Report

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Introduction: Frozen sections have a well-established role in the surgical field. Indications in the liver include: differentiating between benign and malignant hepatic lesions, as well as suspected metastases, incidental findings of a superficial hepatic mass during an operation and assessing suitability of a donor liver transplant. Whilst liver frozen sections are accurate, pitfalls exist. We present a case of a rare liver tumour and its associated diagnostic difficulties in frozen sections.
Case report: A 76 year old gentleman was admitted with malaise and fevers. Investigations showed deranged liver function tests and a large liver lesion with presumed tumour necrosis. When a core biopsy showed reactive changes, frozen sections were undertaken, which revealed nodular fibrotic liver with an area of dysplasia. Permanent sections showed foci of atypical vascular proliferation and the vessels were lined by hyperchromatic cells with an increased nuclear: cytoplasmic ratio. Immunohistochemistry showed positivity for endothelial markers (CD31 and CD34) and a high MIB-1 proliferation index of 80-90%. This was diagnosed as a primary angiosarcoma of the liver, a diagnosis unexpected in the frozen section.
Discussion: Liver frozen sections have a sensitivity and specificity of over 95%; however, there are challenges in this area, which are broadly divided into: sampling errors, sections of poor quality and pathological misinterpretation. Examples of the latter include subtle diagnoses such as lobular carcinoma, as well as malignant mimics, for example von Meyenberg's complex. Furthermore, rare diagnoses such as that of an angiosarcoma require not only prudent sampling, but also provision of full clinic-radiological information.
Conclusion: This case report highlights the issues in frozen sections of rare hepatic tumours. Ultimately, detailed clinical, radiological and serological information are required, as well as vigilance on the histopathologist's part.

P96

Hepatic Angiomyolipoma: A Rare Cause of Hepatic Enlargement

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Purpose of study: We present a case of hepatic angiomyolipoma (HAML) in a 71-year-old female who presented with an incidental finding of an abdominal mass on ultrasound.
Methods and Results: A segment 3 liver wedge containing a tumour was submitted to our histology laboratory. Within the specimen there was a well circumscribed tumour (100x150x80 mm) arising from and contained within the liver. The mass was soft and a relatively homogenous pale cream colour with a vaguely nodular appearance. There was a small cystic area within the tumour. The sections showed a well circumscribed tumour composed of spindle cells in a fascicular arrangement, with interspersed adipose tissue and prominent blood vessels. Megakaryocytes, lymphocytes and myeloprogenitor cells were scattered throughout the lesion. Immunohistochemistry showed patchy positive HMB-45 and SMA staining for the perivascular spindle cells. Megakaryocytes were highlighted with CD61 and myeloprogenitor cells with MPO. The proliferation index (MIB-1) was < 10%. The histological and immunohistochemical profile was in keeping with an angiomyolipoma with extramedullary haematopoiesis.
Discussion: HAML belong to the family of perivascular epithelioid cell tumours (PEComas), that also include pulmonary clear cell sugar tumour and lymphangioleiomyomatosis. HAML is morphologically similar to renal angiomyolipoma and diagnosis is based on the identification of benign smooth muscle cells, fat and blood vessels. The lesions are characteristically immunoreactive for SMA, desmin, S-100 and HMB-45, the latter being the most characteristic. The tumours largely behave benignly, although complications such as haemorrhage can occur; exceptional cases of malignant transformation have been reported.
Summary: HAML are rare mesenchymal tumours composed of fat, smooth muscle and blood vessels.They are immunoreactive for HMB-45 and smooth muscle markers and should be considered in the differential diagnosis of atypical hepatic enlargement.

P97

Echinococcosis (Hydatid Disease): Practical Guidelines for the UK Histopathologist in 2018

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Two main species of the cestode tapeworm, *Echinococcus,* cause significant human disease: *Echinococcus granulosus* is the causative agent of cystic echinococcosis (CE), also known as cystic hydatid disease. *Echinococcus multilocularis* produces alveolar echinococcosis (AE) known as alveolar hydatid disease. Patterns of migration to the UK in the last decade have resulted in both of these diseases being encountered more commonly by surgical histopathologists (particularly those sub-specialising in liver and lung). Despite this, guidelines for the macroscopic and microscopic assessment of these specimens are lacking, resulting in misdiagnoses and incomplete diagnoses, which affect the treatments offered to patients. This poster draws on the experience of a national specialist infectious disease histopathology centre, to offer practical guidance to UK histopathologists in relation to the handling and reporting of hydatid disease specimens. The diagnosis of *Echinococcus granulosus* is often made on imaging, so is provided to the histopathologist on the request form. However, the correct macroscopic handling and sampling of the specimen is crucial in enabling microscopic assessment of viability. Morphologically determined viability underpins the treatment regime, yet this is often not assessed or reported. In contrast, the diagnosis of *Echinococcus multilocularis* is rarely made prior to a histological sample, with a malignancy typically being suspected clinically and radiologically. If left untreated this disease is fatal in >90% of cases and early diagnosis is linked to better prognosis. However, due to lack of awareness of this species, it is invariably misdiagnosed by histopathologists as cystic echinococcosis. In this poster we illustrate the cardinal macroscopic and microscopic features of alveolar echinococcosis and describe the distinguishing features from cystic echinococcosis.

P98

Analysis of Liver Biopsy Referral Practice to a Tertiary Referral Centre in 2015 to Evaluate Whether Specialist Liver Biopsy Reporting Would Benefit Patient Care

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Purpose of the study: Correct clinical management of liver diseases is often heavily reliant on the interpretation of the pathologist examining liver biopsies. Liver pathology is a highly specialised area of pathology that non-specialist pathologists may get insufficient exposure to. We performed a new audit to determine the number of liver biopsies sent to the tertiary referral centre for second opinion/review and evaluate the rate of clinically significant discrepancies between the primary and secondary report. The aim was to assess whether future centralisation of liver biopsy reporting would benefit patient care.
Methods: We identified all medical liver biopsies referred to the tertiary referral centre in 2015 for specialist reporting. We documented the number of medical liver biopsy reviews requested by tertiary centre hepatologists and those received for second opinion from regional histopathologists and recorded the number of cases with diagnostically significant changes at review.
Summary of results: In 2015, a total of 132 medical liver biopsy cases were reviewed at the tertiary referral centre by specialist liver pathologists. Out of these, 42 patients (~30%) had a significant change in diagnosis which would have resulted in altered patient management. The main areas of discrepancy related to biliary diseases, followed by confirmation of autoimmune disease and grading and staging of fatty liver disease.
Conclusion: This audit confirms previous studies in the literature which highlight that liver biopsy review by specialist liver pathologists results in a change in diagnosis or grading/staging of liver disease in a significant proportion of cases. Therefore specialist liver biopsy reporting is advocated as best practice in the management of patients with parenchymal liver disease. This could be achieved by centralisation and/or forming regional networks of pathologists with specialist training in liver pathology.

P99

The Effect of Neoadjuvant Therapy on the Local Immune Signature in Patients with Pancreatic Cancer

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Background: Tumour-infiltrating lymphocytes (TILs) are predictive for response to neoadjuvant chemotherapy in different types of cancer. In pancreatic cancer high tumour infiltration of CD8+ and CD4+ T lymphocytes and low infiltration of T regulatory cells is associated with favourable clinical outcome. However, pancreatic cancer is often characterized by lack of CD8+ T-cell infiltration and by the presence of immunosuppressive myeloid cell populations. It is known that radiotherapy and several forms of chemotherapy can activate the immune system by inducing immune stimulating signals that can increase T cell trafficking to the tumour. The aim of this study is to investigate the effect of the neoadjuvant therapy on the immune profile of the local tumour in pancreatic cancer patients compared to chemo- radiotherapy naïve tumours.
Method: Fresh formalin fixed paraffin embedded (FFPE) tissue samples from resected pancreatic cancer patients in Erasmus Medical Center were used. Gene expression profiling was performed using the nCounter ® PanCancer Immune Profiling panel of NanoString technology. The technique enables the comprehensive multiplex gene expression analysis of 730 genes, presenting 24 different cell types of the immune system. Immunohistochemistry was used for the validation of data.
Results/discussion: The characteristics of the local immune signature and response in pancreatic cancer will be presented and discussed.

P100

BK Virus Nephropathy in Renal Transplant Patients

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Purpose of study: BK virus nephropathy (BKN) is an important cause of renal allograft dysfunction and loss. The emergence of BKN coincided with the use of more potent immunosuppressive agents. Decreased immunosuppression is the principle treatment but predisposes to organ rejection. Here we assess the experience of BKN in a major transplant centre in the U.K.
Methods: Electronic case records of patients diagnosed with BKN in the period October 2014 – September 2017 were reviewed.
Results: 522 renal transplants were undertaken during the study period. There were 18 cases of BKN, giving an incidence of 3.4%. Mean patient age was 57.9 years, and the majority were male (83%). The mean time from transplant to BKN diagnosis was 6.8 months. The average increase in serum creatinine (SCr) at diagnosis was 60.9% from a baseline of the lowest SCr recorded post-transplant. Negative BK virus (BKV) serology post-diagnosis (average time 9 months) was recorded in 50% of patients, who had a mean SCr increase of 144% at most recent follow-up. SCr increase was 212% in patients without documented BKV serum clearance. 12/18 patients (67%) had at least one biopsy post-diagnosis with a BKV negative results in 2/12 (17%). 33% of patients had at least one episode of organ rejection prior to, or concurrently with, BKN diagnosis. 11% had a rejection episode post BKN diagnosis, with a mean 460% SCr increase at most recent follow-up, compared to a 128% SCr increase in those not experiencing a rejection episode post-diagnosis. A single graft was lost 20 months post-diagnosis secondary to medication non-compliance.
Conclusion: The incidence of BKN is similar to that found in comparable study populations. BKN causes a deterioration in graft function and persistent positive BK serology is associated with even poorer graft dysfunction. Subsequent episodes of rejection compound this dysfunction.

P101

Familial Fibrillary Glomerulonephritis with Positive Immunohistochemistry for DNAJB9

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A novel immunohistochemical stain for DNAJB9 has been described as characteristic for fibrillary glomerulonephritis. DNAJB9 is a member of the DNAJ family of chaperones and is involved in endoplasmic reticulum stress and the unfolded protein response. The mechanism by which it accumulates in fibrillary GN is unknown. Cases of familial fibrillary glomerulonephritis have been reported, but are rare. We present the findings in 3 members of a family, all with fibrillary glomerulonephritis. A male (Case 1) was diagnosed in 1984, age 29, with fibrillary glomerulonephritis (clinical indication uncertain). He reached end stage renal failure and was transplanted in 1987, and then again in 2001, with no evidence of recurrent disease. He is currently back on haemodialysis. His sister (Case 2) presented in 2009, aged 43, with severe proteinuria. Her biopsy at that time showed moderate mesangial expansion in glomeruli with extensive deposition of fibrils in the mesangium and in tubular basement membranes, with positive immunohistochemistry for IgG. She has stable CKD stage 3a with minimal proteinuria and is under general nephrology follow-up. This lady's daughter (Case 3) presented in 2016 aged 31 with nephrotic syndrome and deteriorating renal function. The biopsy showed crescents in 4 out of 5 viable glomeruli. On EM the glomeruli contain extensive fibrils 13-26 nm in diameter, with positive immunohistochemistry for predominantly IgG. She was treated with rituximab and steroids, but progressed to end-stage renal failure. She is currently on haemodialysis and being worked-up for renal transplantation. In all cases, Congo red stain was negative. We performed immunohistochemical staining for DNAJB9 and found strong glomerular staining in all 3 cases. In addition, in Case 2, there was tubular basement membrane staining, and in Case1, staining of Bowman's capsule. The findings confirm familial fibrillary glomerulonephritis and we are performing further genetic investigations.

P102

Parosteal Osteosarcoma of the Distal Radius: A Case Report

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Purpose of the study: Parosteal osteosarcoma is an uncommon lesion arising from the cortical surface of bone. It characteristically affects the metaphyseal regions of posterior distal femur, proximal tibia or proximal humerus. Other long bones are rarely involved. We report a case of parosteal osteosarcoma of the left distal radius.
Methods: A 46 year old female presented with a ten month history of left wrist ache associated with a lump. Diagnostic bone core biopsy and imaging investigations indicated parosteal osteosarcoma. Wide local excision was performed and the defect was reconstructed using an osseocutaneous free fibular graft.
Results: The resection specimen demonstrated a solid exophytic tumour measuring 33mm x 24mm x 14 mm confluent with radial cortex. The cut surface was solid, white and bone hard with some peripheral small nodules of neoplastic cartilage. There was no necrosis. Microscopy confirmed characteristic features with mild to moderately atypical spindle cells and well formed parallel trabeculae. There was no evidence of de-differentiation or other high grade features.
Conclusion: Parosteal osteosarcoma is a low grade bone forming neoplasm that has several benign histological and radiological mimics. A high index of suspicion is needed at rare sites. Diagnosis and treatment at a specialist sarcoma centre is recommended.

P103

Tumour Mutational Burden and Mutational Signatures as Biomarkers in Sarcoma

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Aim: To determine if patients with sarcoma could be eligible for immune checkpoint inhibitor therapies based on mutational burden and/or mutational repertoire.
Background: Treatments that enhance the immune system to target cancer have shown promise in recent years with durable responses seen in several tumour types. A high tumour mutational burden is an emerging biomarker of sensitivity to immune checkpoint inhibitors and in some cases is more sensitive than PD-1/PDL1 expression by immunohistochemistry.The use of systemic therapy for metastatic soft tissue sarcomas is controversial with limited options for treatment. The recent FDA approval of immune therapy agents for all solid tumours is therefore prescient.
Methods: Sarcoma exome and genome datasets of 470 bone and soft tissue sarcomas (of multiple subtypes) were obtained (ICGC,TCGA and in-house). Single nucleotide and indel variant calls from benchmarked algorithms were triaged for high quality variants using stringent post-processing filters. These data was interrogated for mutational counts. Mutational signatures were extracted using a non-negative matrix factorisation framework and validated with data from the COSMIC mutational signature database.
Results: Between 2–15% of sarcomas show a high mutational burden depending on the subtype. The mutational repertoire varies between subtypes and is useful for treatment stratification. Interestingly, mutational signature analysis shows that the aetiological spectrum is heterogenous. Up to 5% of hypermutated cases harbour a UV mutational signature. Signatures of DNA repair defects are seen in some sarcomas. Aging patterns also account for a large proportion of mutations (>60%) in many of the sarcoma subtypes. Importantly, there is an overall survival benefit for some patients with an increased mutational burden which is useful preclinical data.
Conclusion: Some sarcomas demonstrate a hypermutator pattern which could be beneficial for clinical trial recruitment.

P104

Fatal Haemoperitoneum, Multifocal Hepatic Kaposi Sarcoma and Systemic HHV-8 Positive, EBV Negative Intravascular Plasmablastic Lymphoproliferative Disorder in a Renal Transplant Patient: A Case Report

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We describe the post-mortem and antemortem findings in a 37-year-old renal transplant male patient who died of intraabdominal hemorrhage following a liver biopsy due to peliosis hepatis arising in HHV8 positive hepatic Kaposi sarcoma. He also had disseminated multiorgan involvement by a peculiar intravascular HHV8 + EBV-plasmablastic lymphoma, not previously reported in the literature. He presented acutely with infective-type symptoms and was managed appropriately with anti-infectives. He continued to deteriorate, requiring ICU level care. Imaging done at that time showed multiple solid liver lesions. A liver biopsy was done and immediately after he became progressively hypotensive, developed hypovolaemic shock and sadly died despite all efforts. At autopsy there was a 2000ml haemoperitoneum. The liver weighed 1650g and was studded with haemorrhagic foci. The rest of the examination was non-contributory. Autopsy liver histolopathology and antemortem liver biopsy showed dilated blood-filled spaces with surrounding and interconnecting vascular lumina associated with a CD31+ HHV8+ spindle cell proliferation, focally forming solid areas. This was regarded as Kaposi sarcoma in the setting of post-transplant immunosuppression. There were also CD20- CD30- MUM1+ OCT2+ BOB1+ PAX5- HHV8+ CD138- EBER-ISH- large lymphoid blasts present in portal tract vessels, scattered in the hepatic sinusoids, and also demonstrated in small vessels in the lung and kidney in the autopsy samples. This case demonstrates a plethora of rare HHV8-driven immunosuppression-related (post-transplant) pathology including systemic, intravascular HHV8+ EBV- plasmablastic lymphoproliferative disorder involving the liver, lung and kidney as well as multifocal hepatic Kaposi sarcoma resulting in peliosis hepatis and fatal abdominal haemorrhage.To our knowledge this is the first case of HHV8+, EBV- systemic intravascular plasmablastic lymphoma in a post-transplant setting described in the literature.

P105

A Helmet-Induced Fracture in Motorcyclists

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Introduction: Post-mortem computed tomography (PMCT) is increasingly being adopted in the investigation of death, usually as an adjunct to autopsy, but increasingly in some countries to replace the internal examination. It has been reported to have an important role in the investigation of trauma due to its ability to investigate skeletal injury. In this context it has been shown to be useful in motorcycle fatalities. We present five cases of a specific skull fracture pattern found in motorcyclists after fatal road traffic collisions using PMCT.
Case series: All individuals suffered a fracture with similar characteristics: a circumferential fracture comprising of a linear fracture of the middle cranial fossa extending through the pituitary fossa, continuing to the temporal and occipital bones of the skull vault. All were wearing full-face helmets and died at the scene due to their head injuries (some combined with injuries sustained elsewhere).
Discussion: Motorcyclists remain some of the most at-risk road users worldwide, and head injury is still the leading cause of death among both helmeted and non-helmeted riders. Despite the clear benefit of wearing a helmet, there appear to be predictable patterns of skull fracture associated with certain types of helmet impacts. Of note all of the deceased had either witness marks on their helmet or injuries indicating an impact to the face. This corresponds with literature which suggests force through the mandibular condyles via the helmet can result in basal skull fractures. However it is thought that skull base and vault fractures can occur with impacts anywhere on the head. Similar fractures appear to have been described in larger studies but lack of detail makes it difficult to confirm.
Conclusion: We suggest that the fractures presented here are distinctly associated with motorcycle incidents. To our knowledge this is the first time the fracture has been described in detail and as a series illustrated using PMCT.

P106

An Analysis of the Causes of Death Identified at Autopsy in the Obese Population

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Purpose of the study: Obesity is the 5th largest risk factor impacting on global mortality and its incidence is rising. Contribution of obesity to death rates is only measurable if included on death certificates. Obesity causes deaths directly e.g. obesity cardiomyopathy, and indirectly as a risk factor for coronary heart disease (CHD) and other conditions. In this study, we investigate the reporting of obesity, and its inclusion in the cause of death formulation, in a single centre coronial autopsy service.
Methods: Retrospective review of autopsy reports in the Oxford pathology database across a 3-year period (2014-16) was performed. Autopsy reports were reviewed for height, weight and BMI, prevalence of obesity and obesity-specific conditions, all-cause mortality, CHD-related mortality, and mean age of death from CHD in different BMI categories.
Summary of results: Height and weight were omitted without adequate reason in 14% of autopsy reports analysed (n= 1,514). Obesity is poorly recognised on death certificates (just 5.1% overall) where present. Identification of OCM in the morbidly obese population is rising; 6.6% compared to 2.0% in the previous largest study. A total of 739 (40%, n= 1,868) autopsies were carried out on obese individuals. Obesity/obesity-specific pathology were included in the cause of death in 0.2% of obese (BMI 30-35), 7.4% of severely obese (BMI 35-40) and 25.7% of morbidly obese (BMI >40) individuals. CHD accounted for 26.4% of deaths in morbidly obese individuals, and 20.7% of deaths in those of ideal BMI. Morbidly obese individuals died from CHD on average 9 years earlier (mean age of death 68 years) compared to those of ideal BMI, mean age of death 77 years (p= 0.000004, 95% CI 5-13); this effect was not accounted for by concurrent presence of diabetes or hypertension.
Conclusions: This study links obesity to earlier death from CHD, and indicates that obesity is under-recorded in the cause of death by pathologists.

P107

Scene Findings Pointing Towards Suspected Suicidal Venous Air Embolism: A Difficult Autopsy Diagnosis to Make

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The routine autopsy rate in the UK is declining, likely as a result of mounting financial pressures and improvements in medical technology making more accurate clinical diagnoses. With the threshold to refer a case for post mortem (PM) examination being raised, there is a higher concentration of more complicated cases coming to the PM room. Whilst the pathology in many of these cases is obvious through thorough examination, other cases are not, and rely heavily on the information coming to the pathologist regarding the circumstances surrounding a death. Here we present one such case, where the autopsy findings provided no obvious cause of death, but retrospective analysis of the scene photographs pointed heavily towards a diagnosis of sudden death secondary to venous air embolism (AE). The photographs showed that the deceased had targeted a large dilated varicosity overlying the saphenous vein, which had collapsed and was no longer visible at the time of autopsy. This case supports a previous audit conducted in our department which evaluated the importance of scene photography in routine coronial practice. One of the key findings of this audit was that scene photography is taken in up to about one third of routine, non-suspicious cases, but this is not always made clear to the pathologist. As a consequence the scene photographs are not viewed and important information concerning the scene can be missed. With conflicting opinions in the literature, diagnosing an AE at autopsy remains challenging. We discuss the promises and pitfalls of radiological versus dissection techniques, but suggest that having a high index of suspicion for AE is centrally important. This is of particular value where the cause of death appears obscure or where there are incised wounds to the skin that could be associated with venous damage.

P108

A Quarter Century of Decline of Autopsies in the Netherlands

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Objective: Even in this modern era autopsies provide invaluable information on the correct cause of death, and contribute greatly to quality assurance. Controversially, autopsy rates have been declining worldwide. The aim of this retrospective study was to study the trend of all hospital autopsies in the Netherlands over a course of 25 years, as well as to investigate autopsy rate variations due to aging, sex, the cause of death, and a history of cancer.
Method: Retrospectively, 25 years of data on clinical autopsies from the Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands (PALGA) was paired with the cause of death registry maintained by Statistics Netherlands. Next, the Netherlands Cohort Study (NLCS) was linked with PALGA, the Dutch Population Register (GBA) and Statistics Netherlands.
Results: The prevalence of autopsies declined from 7,07% in 1991 to 2,73% in 2015. While sex did not seem to affect autopsy rate, increasing age significantly decreased the rate of autopsies. Clinical cause of death was obtained for 59,760 deceased in a period from 1991-2009, which showed an enormous variation in the autopsy rate. The highest autopsy rate was observed in deaths related to the digestive system, while the lowest was observed in deaths related to mental and behavioral diseases. Furthermore, the more coded causes of death, the higher the autopsy rate. Lastly, presence of cancer only showed an increased autopsy rate in the first year after diagnosis.
Conclusion: Over the last quarter century autopsies in the Netherlands have declined. The gravest decline as well as the lowest autopsy rate was observed in people with older age. In contrast to other authors, sex does not seem to affect the autopsy rate, when differences in age distribution at death are adjusted for. Multiple contributing causes of death increased the autopsy rate, whereas a presence of cancer only showed an increased autopsy rate in the first year after diagnosis.

P109

Characterization of Human Endometrial and Placental Organoid as an In Vitro System to Study Fetal-Maternal Interaction During Early Pregnancy

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There is currently limited physiologically relevant systems to investigate common conditions that have a major impact on women's quality of life, such as endometriosis and endometrial cancer. Previous studies have been done using mouse models as a basis of research, however, mouse endometrium is different to human endometrium so these trials had limited success. We conducted a comprehensive review of commercially and academically available culture systems and found that there were no existing systems which both, sustain growth and recapitulate endometrial function in vitro. We established a system using adult stem-cell derived organoid cultures from several human organs to generate 3D cultures of human endometrium. We successfully established these organoids using endometrial biopsies and optimized culture conditions through methods such as tissue culturing and immunohistochemistry. These endometrial organoids recapitulate many features of uterine glands in vivo such as the ability to respond to hormonal signals and their ability to differentiate into ciliated and secretory epithelial cells. These organoids functionally respond to the sex hormones estrogen and progesterone and mimic the cyclical changes of the glands during the menstrual cycle as shown by their proliferative and differentiation response in vitro. They are capable of long-term expansion and are genetically stable. This human endometrial organoid culture will be a useful tool for the investigation of the physiology of early human pregnancy, endometriosis and endometrial cancer as well as investigating the interaction between the trophoblast of the developing conceptus and uterine glands.

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P111

Can We Predict Radiosensitivity in Non-Small Cell Lung Cancer?

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The aim is to identify histopathological features which may predict radiosensitivity in patients with NSCLC.

Materials and methods: We have identified a set of 67 Leicester NSCLC cases for which pre-treatment archival tissue is available, there is a history of radiotherapy, and CT imaging follow-up from the period 2009 to 2014. Digital images of archival diagnostic tissue sections were examined to derive morphological measures with the potential to predict radiosensitivity. Quantitative radiological measures of response up to 6 months after radiotherapy were derived by specialist radiological examination of imaging. we standardised by calculating the fraction residual tumour at 100 days after radiotherapy(FRT100).

Results: At present, 67 lung cancer samples have been enrolled. 31(46.3%) are SqCC/ favouring SqCC and 36 (53.7%) are ACA/ favouring ACA. FRT100 is higher in patients with multipolar mitosis (p=0.03*), and lower in cases showing a NE dif. (p= 0.05*). FRT100 shows a relationship with multipolar mitosis (R 2 = 0.14, p=0.005*) and negative relationship with neuroendocrine (NE) dif. (R 2 =0.06, p=0.058). Patients with good response after RTx had higher survival percentage during the first 3 years, after that period the tendency of both groups tend to be similar (p= 0.12). Patients with SqCC/ favouring SqCC had a poor prognosis, because nobody was alive after 4 years, while 21.8% of the ACA were still alive after 4 years (p= 0.04*). Patients with multipolar mitosis had a poor prognosis because they die within 3 years after RTx (p= 0.02*). Finally, there was no association between NE dif., and overall survival (p=0.21).

Conclusions: Multipolar mitosis and neuroendocrine differentiation may be predictive histological markers of radiosensitivity in NSCLC. FRT100 initially predicts survival well, but the prognostic value quickly diminishes as tumours eventually progress.In our cohort SqCC has shown a poor prognosis in comparison with ACA.

P112

An Improved Monolayer Culture Technique for Driving Mouse Embryonic Stem Cells to Intestinal Differentiation

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Mouse embryonic stem cells (mESC) are a useful surrogate for human pluripotent stem cells for use in in vitro assays, as there are many similarities between the differentiation of mouse and human pluripotent stem cells. One of the challenges in culturing mESC is their tendency to grow as aggregates of a small number of cells (embryoid bodies) rather than as monolayers. The use of embryoid bodies makes reliably controlling the differentiation of stem cells difficult, and monolayer techniques should improve the yield and homogeneity of differentiated cells. However, the efficiency of monolayer techniques for driving differentiation has been poor traditionally. The aim of this study was to develop an improved monolayer technique for driving intestinal differentiation from mESC. mESC were grown as monolayers on culture plates in basic medium supplemented with recombinant Activin A, either recombinant Wnt-3a or CHIR-99021 (an activator of beta-catenin signalling) and with or without recombinant Noggin for four days, to generate definitive endoderm. Cells were then treated with basic medium containing CHIR-99021 for 6 days to promote intestinal differentiation. The generated cells were characterised by immunocytochemistry and qPCR for endodermal and intestinal epithelial markers. The cells were grown in an organotypic matrix culture system and characterised for their ability to retain expression of intestinal markers and form crypt-like structures. A combination of Activin A, Wnt-3a and Noggin supplementation in culture medium, followed by CHIR-99021 led to the highest levels of persistent expression of intestinal epithelial markers and formation of intestinal crypt-like structures. We have developed a method for efficiently and reliably generating posterior endoderm / intestinal epithelium from mESC using monolayer cultures. This technique has advantages over existing monolayer and embryoid body techniques.

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P113

A New Forced Aggregation Method for Generating Embryoid Bodies from Mouse Embryonic Stem Cells

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Embryonic stem cells (ESC) have the ability to develop into many types of differentiated tissues if they are placed into a differentiating environment. A common method to induce in vitro differentiation of ESC is through formation of an aggregate of a small number of stem cells into an embryoid body (EB). EBs mimic the initial stages of differentiation in ESC and can be manipulated to induce specific cell lineages. Several techniques exist for generating EB, such as the hanging drop technique, however, it can be difficult to standardise formation of EB using existing methods. Here we describe a new cost-effective method using forced aggregation on ultra-low attachment 96-well plates to form EB from mouse ESC. Mouse ESC were dissociated and transferred to a 96-well plate ultra-low attachment plate, such that there were approximately 500 cells per well. Plates were centrifuged to force aggregation. This was compared against a hanging drop method with the same cell numbers. A further comparison of media (DMEM) with calf serum vs. knockout serum replacement media was performed. EB generated using all methods were grown for 5 days and then compared by computer-assisted brightfield microscopy for survival, roundness and area. The forced aggregation method led to approximately 90% EB survival at 5 days, compared to 50% by hanging drop, and less variability in size and roundness compared to the hanging drop method. Serum replacement media led to slightly smaller EB ($p<0.001$) compared with media containing calf serum in both methods; other parameters were not significantly different. This forced aggregation method with knockout serum-replacement media is capable of generating a high yield of standardised EB. The smaller EB generated by serum-free media are likely to be more responsive to exogenous growth factors. This offers an improvement over existing techniques, with optimised media constituents for supporting EB growth.

Research supported by PathSoc Small Grant Scheme.

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P116

Involvement of Cten in TGFß1-Driven EMT in Colorectal Cell Lines

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Epithelial to mesenchymal transition (EMT) is an early event in the metastatic process. We have investigated the involvement of Cten (C-terminal tensin, TNS4) in the EMT upon TGF ß1stimulation. Human colorectal cancer cell lines (SW620, SW620ΔCten, HCT116, SW480) were treated with recombinant human TGFß1 (20ng/mL, 48h) after overnight starvation. The effects of TGFß1 stimulation in TNS4 signalling and EMT were assessed by WB, immunofluorescence and functional assays on migration, invasion and cell viability. The effect of TGF-β1 on Cten and its downstream targets was again investigated in SW620 by transient siRNA knockdown by and changes in protein expression level of Cten, ROCK1, Src, Snail, E-cadherin and N-cadherin were confirmed by western blot. Cten and Snail upregulation was detected after TGFß1 stimulation, together with an increase in its nuclear translocation and upregulation of Src/ROCK1/Snail signalling pathways. Co-localization of Cten with ITGB1 and Snail was also detected by confocal microscopy. A Cten Knockout does not impact TGF-β1-induced cell proliferation but abrogates TGF-β1 induced cell migration and invasion. TGFβ1 and Cten signalling pathways seem to cooperate in promoting EMT and early metastasis in CRC cells through the dependent upregulation of the Src/ROCK1/Snail signalling axis. If these preliminary results are confirmed, Cten may become a potential therapeutic target for the prevention of metastasis in colorectal cancer.

P117

Characterization of the Immune Infiltrate Present in Early and Late Stage Sporadic and Hereditary Neoplastic Lesions of the Colon

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Purpose of the study: To determine whether immune infiltration of early and late stage neoplastic lesions varies in the context of sporadic and hereditary development of colorectal adenomas.
Methods: 14 patients with neoplastic lesions of the colon were selected from the tumour bank. Nine were familial adenomatous polyposis (FAP) patients and 5 were sporadic colorectal cancer (CRC) patients. Immunohistochemistry was performed for CD3+, CD4+, CD8+, CD57+, CD68+ and FoxP3+ to determine the density of infiltration of immune cells for each lesion (normal, low-grade dysplasia, high-grade dysplasia and adenocarcinoma) and location (stromal [s] or intratumoural [t]). The immune reactive positive lymphocytes were manually counted using Image J in an area of 1 mm2 (4 images of high density spots at 400x magnification).
Summary of results: The frequency of CD8 lymphocytes in the stroma of normal tissue (p=0.039), CD68 cells in low-grade dysplasia (p=0.031), high-grade dysplasia (p=0.020) and adenocarcinoma (p=0.034) and FoxP3 lymphocytes in all lesions (normal p=0.011, low-grade dysplasia p=0.013, high-grade dysplasia p=0.019, adenocarcinoma p=0.034) were significantly higher in the sporadic patient group than in the FAP patient group. In all cases, in both patients groups, the amount of infiltrating immune cells was higher in the stroma (s) than in the tumour (t).
Conclusions: The data indicates a tendency towards a higher density of immune cells in sporadic CRC cases than FAP cases. CD8 T cells, macrophages and regulatory T cells (T-regs) may be important cells in determining differential activity of the immune system towards neoplastic lesions in the sporadic and FAP contexts. However, this finding needs to be validated in a larger sample size and using an alternative model.

P118

Celebrating a Century of Women in Pathology

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The UK Royal College of Pathologists recently celebrated International Women's Day, reporting that 47% of UK pathology consultants are now female. In this centenary year of limited women's suffrage in the UK, this study assesses the careers of early women pathologists in Glasgow (1910-1930) using contemporary archives and details in Medical Registers and Directories. Women were barred from studying or working in the Western Infirmary, famed for the academic Pathology Department led by Professor Sir Robert Muir. Instead, women students attended Glasgow Royal Infirmary (GRI), whose Pathology Department was led by Professor John Teacher. Within the GRI Pathology Department 20 of 38 "assistants" over the period 1910-1930 were women. Comparison of the women's later careers with those of the men assistants (excluding 1 man killed in the War) shows that women were less likely to achieve higher qualifications (6/20 women, 10/17 men), to report scientific papers (5/20 women reporting mean 2.4 papers, 10/17 men reporting mean 4.5 papers), and to achieve career progression. Five women became long-term assistants, 3 within the GRI Department, but 2 men became Professors of Pathology. Four men, but no women, became Professors in other specialties. Comparison with published reports about women doctors of the era shows that the women pathology assistants were atypical. Only 1 entered general practice and 1 mission work, but 4 entered surgery, including one who founded hospitals in Serbia after wartime service with the Scottish Women's Hospitals. This study suggests that women, excluded from the prestigious Western Infirmary, were accepted in the Pathology Department at GRI by Professor Teacher. Their later careers were limited by opportunities available then to women.

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Audit on the Turnaround Time Reporting of Cervical Biopsy at a Tertiary Care Hospital

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Purpose of Study: NHS Cervical Screening programme (NHSCSP) is one of the successful screening programs in the world and histopathology reporting plays a vital role as the biopsies are the gold standard against which cytological and colposcopic findings are co-related. Hence it's of paramount importance that the turnaround time (TAT) of reporting cervical biopsies is audited regularly and reasons for delays explored, for the efficient functioning of the screening program. According to NHSCSP document 10 and RCPath key performance indicators; 80% of the cases would be reported within 7 calendar days and 90% of all cases are reported within 10 calendar days
Objectives: 1.To compare the reporting TAT of cervical biopsy specimens to the NHSCSP- KPI standards. 2.To compare the results with the previous audit conducted in 2013 (Jan–March)
Methods: The biopsies received in the department from 01/04/2017 to 30/06/2017 were retrieved from the computer assisted search using the SNOMED codes for Cervix and Gynaecological biopsy. The TAT was calculated as calendar days from the receipt of the specimen to the authorisation of reports.
Summary of results: 274 biopsies were retrieved using this method.9 cases were excluded(n=265).The average turnaround time was 5.9 calendar days (Range: 2-23 days) in comparison to 6.03 calendar days in 2013.The TAT was ≤7 days in 221 cases (83%) compared to 233 cases (74%) in 2013. The TAT was >7 days in 44 cases (17%).The TAT was <10 days in 249 cases (93%).The reasons behind the delays were also looked into and showed that in 14 cases (32%) extra levels and immunohistochemistry was required, 6 cases (14%) were delayed because of long bank holiday weekend and no explanation could be found in 24 cases (54%)
Conclusion: The TAT was compliant with KPI standards in the current audit with reporting of 83% (80%) cases and 93% (90%) cases within 7 and 10 days respectively.

P122

The Proportion of Older Men with Testicular Germ Cell Tumours is Increasing: Epidemiological Observations from a Large Cohort 1991–2017

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Objectives: Testicular cancer is the most common solid malignancy to affect young men. Testicular germ cell tumours (GCTs), seminoma (SGCT), and non-seminoma (NSGCTs) represent the majority. Anecdotal MDT observations suggest that the number of older men presenting with GCTs is increasing. We determined whether the age of patients at presentation with GCTs is changing within our population.
Methods: A pathology departmental database search identified 2933 orchidectomy specimens between 1991 and 2017. Review of reports identified 623 GCTs (age range 15–77). Historic cases were reclassified using WHO terminology and pT staged. Patients were grouped according to year of presentation (1991–1999, 2000–2008 and 2009–2017) and mean age was compared using SPSS ® analysis of variance. The proportion of men aged ≥50 or with pT3 disease was compared using the Chi-squared test.
Results: The mean age of patients presenting with GCTs increased significantly, from 35 to 39 years ($p<0.01$). An increase was observed for both SGCT ($p<0.01$) and NSGCTs (<0.05). The mean age of patients presenting with testicular lymphoma has not significantly changed during this time (n=32, p=0.24). The proportion of men presenting with GCTs aged ≥50 has increased from 7% to 17% ($p<0.01$) and the proportion presenting with pT3 disease has also increased with time from 5% to 11% ($p<0.05$).
Conclusion: We observe a significant increase in the proportion of older men presenting with GCTs. This change appears to be specific to GCTs because the mean age of patients presenting with testicular lymphoma has not changed significantly. The aetiology of this change remains unclear, but the concomitant increase in the proportion of higher stage disease could suggest either increased frequency of delayed presentations or that the disease is more aggressive in older men. These observations may have clinical implications for the oncological treatment of this older population.

P123

Is p63 Involved in Cancer Stem Cells and Metastasis in Prostate Cancer?

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Purpose of the study: p63 and more precisely the ΔNp63 isoform is a widely used marker of basal or myoepithelial cells including prostate and breast. It was also proposed as a master regulator of prostate stem cell regulation and self-renewal. Most recently, p63 has been implicated in regulating prostate cancer metastasis. Therefore, we investigated the occurrence of p63 in a set of lymph-node metastatic prostate tissues.
Methods: We used immunohistochemistry to study p63, p63 isoforms and markers of differentiation in lymph nodes from locally metastatic prostate cancers.
Summary of Results: We found that 3 from 43 patient samples contained a small sub-population of p63-positive cells. These cells were positive with an isoform-specific ΔNp63 monoclonal antibody, but were not positive for the TAp63 isoform. We further examined the phenotype of the ΔNp63+ cells using co-localisation with cytokeratins, differentiation markers and potential markers of prostate stem cells. ΔNp63 positive cells were negative or mostly negative for the cancer stem cell markers ALDH1 and CD44. ΔNp63+ cells are cytokeratin positive using AE1/AE3 and are variably positive for basal cytokeratins, but negative for cytokeratin 8. These cells also express androgen receptor.
Conclusions: These data indicate that ΔNp63 is rarely expressed in prostate cancer metastases, contrary to recent publications indicating a specific role in the metastatic process. It is also unlikely that ΔNp63 represents a general marker of a cancer stem cell population in prostate cancer, despite playing a role in normal prostate development.
Acknowledgement: The work was supported by the project MEYS - NPS I - LO1413 and by the Czech Science Foundation (Project no. P206/12/G151). MG is Brno Ph.D. Talent Scholarship Holder – Funded by the Brno City Municipality.

P124

Development of a Prognostic Risk Model by Systematic Evaluation of Prognostic Methylation Markers for Renal Cell Carcinoma

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Background: Current prognostic models are unable to accurately identify renal cell carcinoma (RCC) patients at high risk of metastasis or recurrence. As 5-year survival rates range between 8–12%, improving these models is necessary for better patient management.
Methods: Formalin-fixed paraffin-embedded tissue samples from 336 non-metastatic clear-cell RCC patients were selected from the Netherlands Cohort Study (NLCS). Promoter methylation of *PCDH8, BNC1, SCUBE3, GREM1, LAD1, NEFH, RASSF1A, GATA5, SFRP1, CDO1* and *NEURL* was determined with nested methylation-specific PCR. To identify clinically relevant methylated regions, The Cancer Genome Atlas (TCGA) was used to guide primer design. A Cox proportional hazards model containing clinical factors (age at diagnosis, sex, TNM stage, Fuhrman grade and tumour size) and biomarkers was developed using stepwise backward elimination. To correct for optimism, internal validation using bootstrapping was done.
Results: Methylation of six biomarkers (*GREM1, GATA5, LAD1, NEFH, NEURL* and *LAD1*) was associated with poor ccRCC-specific survival, independent from clinical factors. The final model contained clinical factors and five markers (*GREM1, GATA5, NEFH, NEURL* and *LAD1*); c-statistic 0.71 (0.65 after correction for optimism), Akaike Information Criterion (AIC) 675. Model fit and performance were better in the biomarker model as compared to the model with clinical factors only (c-statistic 0.66; AIC 1074). Validation in TCGA however, showed comparable results for models with and without biomarkers (c-statistic 0.76 AIC 475 and c-statistic 0.75 AIC 470 respectively).
Conclusion: This study reports on five biomarkers that can be added to current models to better identify ccRCC patients with a poor prognosis. In addition, we show that using TCGA to guide primer design is a good approach to identify clinically relevant methylated regions.

P125

Diagnostic DNA Methylation Markers for Renal Cell Carcinoma: A Systematic Review

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Background: As the 5-year survival of metastasized renal cell carcinoma (RCC) is only 12% but vastly increases in less advanced disease stages, early RCC diagnosis is crucial to increase survival rates. Over the last years, many methylation biomarkers have been suggested as diagnostic markers for RCC. However, the current state of clinical translation of these markers is unclear and a comprehensive overview is urgently needed.
Methods: We systematically included 19 articles obtained through PubMed, EMBASE, Medline and Google Scholar searches, reporting on 44 individual methylation markers. Included studies were scored according to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) criteria. Forest plots were used to visualize and summarize diagnostic performance of all studied markers.
Results: A total of 44 different markers and 11 multigene panels were identified, but only 16 markers (36%) were independently validated. Markers were measured in tumour tissue (N=12; 63%), blood (N=3; 16%), tissue and urine (N=3; 16%) and blood and urine (N=1; 5%). STARD scores for the 19 studies varied from 4-13 out of the maximum 23 points, with a median of 10 points. A wide variation in subgroups, methods and primer locations was observed. Ten markers were consistently associated with RCC diagnosis, however with low sensitivities ranging from 1-66%. None of the 44 markers exceeded Level of Evidence III.
Conclusion: This systematic review provides an overview of diagnostic methylation markers for RCC and their current Level of Evidence, and indicates that none of these markers are ready for clinical translation yet. In addition, the majority of studies lack good reporting quality according to the STARD criteria, thereby hampering the development of diagnostic molecular biomarkers for RCC.

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This abstract has been withdrawn

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Effect of Smoking on Different Histotypes of Kidney and Urinary Tract Cancer in Women

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Introduction: It is known that smoking is associated with an increased risk of cancers of the kidney and urinary tract, but few epidemiological studies have sufficient cases to explore heterogeneity between histological tumour types and sites.
Methods: Study participants completed a questionnaire on reproductive, anthropometric, and lifestyle factors, including smoking, at recruitment in 1996–2001, and were followed for cancer and death via national registries. Using Cox regression models, we estimated relative risks (RRs) of cancers of the kidney and urinary tract associated with current versus never smokers, adjusted for potential confounders.
Results: In 1,242,530 women without previous cancer, 4251 cancers of the kidney (including renal pelvis) had accrued after an average of 15.4 years of follow-up. Smoking was associated with an increased risk of kidney cancer, but this differed significantly by tumour histotype. Current versus never smoking was associated with a 50% increased risk of renal cell carcinomas (RCCs, n=3271; RR=1.54, 95% CI: 1.41-1.68), and a larger, three-fold risk, of transitional cell carcinomas (TCCs, n=330; RR=3.34, 95% CI: 2.56-4.35); heterogeneity by histotype, p<0.0001. Current smoking was also associated with some 3-4 fold increased risks of TCCs of the ureter (n=185; RR=4.33, 95% CI: 3.03-6.20) and bladder (n=2344; RR=3.29, 95% CI: 2.97-3.63).
Conclusions: While smoking is known to increase the risk of renal cancer, there are substantial differences in the magnitude of the risk by histotype. TCCs in different parts of the urinary tract show consistently stronger associations with smoking than RCCs.

P128

Pathologist Assessment of Novel Histological Features in Prostate Cancer

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Purpose of the study: Current methods of stratification for treatment and prognostication of prostate cancer need urgent improvement. As part of the work up to establishing an image analysis pipeline for prostate cancer, a study was undertaken to evaluate the feasibility and limitations to pathologist assessment of novel histological features. Features were chosen that have been shown to be important for outcome or are linked to specific molecular changes in prostate cancer or other tumour types.
Methods: A cohort of 27 formalin-fixed paraffin embedded radical prostatectomy specimens was utilised where next generation DNA sequencing data was available from a novel in-house prostate cancer panel. The features chosen to be assessed by 2 independent pathology observers included percentage stroma content within the tumour area, percentage gland formation, stroma texture, tumour infiltrating lymphocyte content, nuclear size and nucleolar prominence.
Summary of results: Significant limitations in manual analysis of features was identified including assessments being time consuming with poor inter-observer reproducibility. Features such as nucleolar prominence were too complex to be assessed. Interobserver kappa levels of agreement were minimal to weak only, for example, 0.34 (stroma texture). Some trends in association with molecular aberrations were seen, such as copy number changes in FOXA1 being linked with low stroma content.
Conclusions: Manual assessment of novel histological features in prostate cancer by pathologists is inherently limited by the manual assessments that can be made by eye and inter-observer variability in assessing more complex features. Deep learning image analysis approaches offer the opportunity to overcome these limitations.

P129

Malignant Rhaboid Tumour Case Report: Importance of Cytogenetics and SALL4 Immunohistochemistry

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Case presentation: A one year old male infant with a history of chronic constipation was admitted for acute intestinal obstruction. The abdominal MRI identified a large abdominal mass (5.6 cm). Histology showed a highly mitotic epithelioid neoplasia composed of cohesive cells with eosinophilic cytoplasm. The vesicular nuclei presented a prominent nucleolus. There were no typical rhabdoid features to be observed. However, immunohistochemistry was typical with a strong EMA positivity, focal keratin positivity and a complete loss of INI-1 expression. SALL4 was positive, and all other germinal cell markers were negative (Glypican-3, beta-HCG, OCT3a and alfa-FP). The final diagnosis was concluded to be an extra-renal malignant rhaboid tumour. The molecular karyotype (CGH60K) showed a simple karyotype with only a 6p22del and a 22q11del with homozygous deletion of SMARCB1.
Discussion: INI-1 loss is a consequence of this bi-allelic inactivation of SMARCB1. INI-1 is a member of the SWI/SNF chromatin-remodelling complex. It is thought to be the main oncogenic driver as it plays a critical role in epigenetic regulation affecting the whole genome. Consequently, it can dysregulate many pathways and cell cycle cascades. It is to be noted that such a broad impact on the genome regulation can result in an aggressive tumour behaviour without complex genomic rearrangements. In adults the loss of expression of INI-1 has been observed in various other tumours. A difficult differential diagnosis can happen between MRT and an epitheloid sarcoma (ES), since they both might have rhabdoid features, a loss of INI-1 expression and a simple karyotype (especially in classical/peripheral ES). A study showed that SALL4 may aid in the differential diagnosis between MRT (67% SALL4+ (n=15)) and ES (3% SALL4+ (n=36)).

P130

Differential Morphometric Placental Villous and Vascular Characteristics in Early- and Late-Onset Placental Syndrome

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Preeclampsia (PE) and fetal growth restriction (FGR) are common complications of pregnancy. We investigated villous and vascular growth in placentas of women with and without PE/FGR using a novel method of morphometric analysis of placenta tissue obtained in routine clinical setting. Fifty-seven placentas of various gestational age (GA) were morphometrically investigated on villus area, vessel area, lumen area and vessel number. In addition, the degree of villous capillarisation was assessed by total vessel area/villus area and total vessel lumen area/villus area. Gestational age (before or after 34 weeks) was taken into account (n=29 early; n=28 late) as well as with PE/FGR (n=38) or not (n=19). Groups were divided by gestational age as well as by clinical phenotype. Sub-analyses were performed based on umbilical artery Doppler velocimetry data in PE/FGR. We demonstrated reduced villus area in PE/FGR compared to No PE/FGR, with a significant interaction effect of GA (larger effect in early-onset versus late-onset), while capillarisation was not significantly affected by GA. Early-onset Delivery placentas showed less capillarisation than Late-onset Delivery (irrespective of PE/FGR). Interestingly, vascularisation data show a gradually increasing scale with decreasing umbilical artery PI and RI. Our results indicate that GA affects placental morphometry with a differential effect on villous and microvascular development. We also show abnormal villous growth in early-onset PE/FGR and normal growth in late-onset PE/FGR, confirming these are separate entities wtih different etiologies. Our morphometrical approach can be used in larger patient cohorts to investigate villous/microvascular interactions in these important obstetrical syndromes.

P131

Extramedullary Haematopoiesis Presenting as Anterior Neck Mass: A Systemic Review of Two Cases

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Background: Extramedullary haematopoiesis (EH) is haematopoiesis occurring in organs outside the bone marrow. It normally occurs in the spleen, liver and lymph nodes. This process is essential during fetal life but its occurrence after birth is considered pathological. The spleen and liver are the usual site for pathological EH, but other areas including the CNS, pericardium, GIT, thyroid, breast and prostate have been reported.
Methods: We report two cases of EH presenting as anterior neck mass in pediatric age group. The mass both measured 3x2x1cm. Excised mass was stained with H&E stains and Hb electrophoresis was performed on patient blood sample for haemoglobinopathies.
Results: Both patients were female aged 2 and 4 years respectively. They both presented with anterior neck swelling 3 and 6 months after birth, no stigmata of haemogobinopathy. Microscopically, haematopoietic cells containing all the three cell lineages with nucleated red blood cells seen in areas. Also seen are sickled red blood cells in the two year old patient biopsy. Hb electrophoresis was positive (SS) in that patient and negative in the four year old.
Conclusion: EH is a rare entity but it should be kept in mind as a possible cause of anterior neck mass. Hemogobinonopathy should be rule out in our environment; in some cases no etiological factor can be identified.

P132

Tumour Infiltrating Lymphocytes and CD4/FOXP3 Ratios Predicts Survival Using Digital Image Analysis

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Background: Tumour infiltrating lymphocytes (TILs) have been associated with improved prognosis in oropharyngeal squamous cell carcinoma (OPSCC); however, the survival benefit appears modest and favours HPV positive OPSCC. The current literature presents a mixed view on the best method to score TILs. This study investigates the prognostic value of assessing TILs by H&E in HNSCC using digital pathology solutions against human eye visual assessment and by comparing TIL estimates in H&Es against markers of adaptive immunity (CD3, CD4,CD8 and FOXP3) using digital image analysis.
Methods: This study utilised 190 OPSCC in TMAs. These TMAs were stained with either H&E or IHC for CD3, CD4, CD8 and FOXP3. Visual estimates of TIL counts by human eye from H&E stained slides were assessed on the whole core at x10 magnification based on published thresholds. Image analysis was performed on whole cores using image analysis software QuPath on H&E and IHC stained slides. HPV status was determined by p16 IHC and HPV RNAScope.
Results: Scoring of TILs in H&Es by visual estimates was prone to misestimation and reduced survival estimates when using predetermined thresholds compared to machine scoring by image analysis. Comparison of digital pathology solutions assessing the TIL population in H&E and IHC stained slides found the percentage of TILs present to be significantly higher in the HPV positive's compared to the HPV negative's in the H&E, CD3 and CD8 immunostained slides but not CD4 or FOXP3. When dichotomised into high and low TILs only H&E based TIL counts and CD4/FOXP3 ratios were found to be independently predictive of survival when adjusted for age and smoking history.
Conclusion: Image analysis creates continuous variables from which TIL counts can be reliably assessed. The adaptive immune landscape of HPV negative OPSCC is significantly different to HPV positive OPSCC of which only total TIL counts and CD4/FOXP3 ratios appears to be predictive of five-year survival.

P133

PD-L1 (Programmed Death-Ligand 1) Expression is Associated with Increased CD8+ Tumour-Infiltrating Lymphocytes (TILs) in Oropharyngeal Squamous Cell Carcinoma (OPSCC)

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Purpose: PD-L1 is a protein that can be expressed as part of an immune surveillance evasion mechanism in various cancers including OPSCC. We analysed the expression of PD-L1 by IHC and its clinical-pathologic associations in a cohort of 65 Irish patients with OPSCC presenting over a 15 year time period.
Methods: Clinical-pathologic data included age, sex, primary subsite within the oropharynx, stage of tumour, requirement for percutaneous endoscopic gastrostomy (PEG), radiologically inserted percutaneous gastrostomy (RIG) tube or tracheostomy and survival. We analysed H&E, p16, p53, CD8 and PD-L1. Manual estimation of the percentage of tumour cells expressing PD-L1 and the percentage of cells in the tumour parenchyma being CD8+ TILs were made. We defined PD-L1 as positive when ≥5% of tumour cells expressed any intensity of membraneous staining, tumours to have increased CD8+ TILs when ≥5% of all cells within the tumour parenchyma were CD8+ TILs, and assessed p16 using a ≥70% cut-off, all of which are methods utilised in recent studies.
Results: 25/65 (38.5%) were p16+, 33/65 (50.8%) were PD-L1+ and 17/65 (26.2%) had increased CD8+ TILs. There was a significant positive correlation seen between the percentage positivity of PD-L1 in tumour cells and percentage of CD8+ TILs (p=0.0002, Spearman rho 0.445). There was a significant association between increased CD8+ TILs and p16 positivity (p=0.001). There was a trend demonstrated between p16 and PDL-1 expression (p=0.092). We also noted increased staining for PD-L1 at the periphery of tumours in many cases.
Conclusion: We found significant positive correlations between both PD-L1 expression and increased CD8+ TILs and between p16+ tumours and increased CD8+ TILs and a trend for p16+ tumours to show increased PD-L1 expression. Our findings support our current understanding that PD-L1 expression is an immune surveillance evasion mechanism employed by OPSCCs, and that they may respond to treatment targeted towards PD-L1

P134

A High Density of CD45-Positive Lymphocytes in Resection Margins of Primary OSCC Predicts Local Recurrence

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The local recurrence rate of oral SCCs (OSCCs) hardly decreases. This is partly due to the presence of (pre)malignant cells in the remaining tissue after resection, which may lead to the development of a new tumour in time. Because histopathologic detection of (pre)malignant cells in tumour resection margins is unreliable to recognize patients at risk for recurrence, we determined if CD45, CD8 and/or PD-L1 expression in OSCC and/or the adjacent resection margins may be used for this purpose. FFPE tissue sections of 41 primary OSCC, the histopathologically confirmed tumour-free resection margins and 11 recurrences were analyzed. Inclusion criteria comprised a minimal 5y follow-up period and surgery without adjuvant radio- and/or chemotherapy. Immunohistochemistry was performed using CD45-, CD8- and PD-L1-specific (clone 22C3) antibodies on a Dako Autostainer Link 48. Immunostaining revealed that 1) a low density of CD45-positive lymphocytes (<3.54%/0.977mm2) in OSCCs is associated with recurrence (p=0.03), 2) a high density of both CD45- and CD8-positive lymphocytes (≥7.72% and ≥4.77%, respectively) just below the squamous epithelium in tumour resection margins correlated with recurrence (p=0.001 and p=0.003, respectively), 3) CD45 combined with chromosomal instability (Pierssens et al., Oral Oncology 2017) in tumour resection margins is the most optimal predictor for recurrence (p<0.001), 4) neither tumour PD-L1 expression nor histopathologic classification correlates with recurrence. Both a high density of CD45- and CD8-positive lymphocytes alone, or (CD45) combined with chromosomal instability in tumour resection margins correlated with unfavourable recurrence-free survival (p≤0.002). A low density of CD45-positive lymphocytes in OSCC or a high density in tumour resection margins, either alone or in combination with chromosomal instability, can reliably identify patients at risk for developing a local recurrence.

P135

Extramammary Paget's Disease: A Clinicopathological Study of 31 Cases

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Background: Extramammary Paget's disease (EMPD) is an uncommon cutaneous neoplasm mainly affecting the anogenital region of elderly patients. EMPD can be primary, of cutaneous origin, or secondary, arising from a visceral malignancy, most often of an anorectal or urogenital origin. Most primary EMPD are in-situ although invasive adenocarcinoma arising from EMPD is a rare but known phenomenon.
Method: We have performed a retrospective analysis of all the cases of EMPD in our centre. We reviewed the pathology report and extracted relevant clinical, pathological and staging information. 35 cases of EMPD were identified, 4 of which were secondary EMPD and therefore excluded from assessment.
Result: Of the 31 cases of primary EMPD identified, 13 had invasive disease. Nearly half of these patients were diagnosed with invasive adenocarcinoma on their first biopsy. 8 of the patients were male and most cases presented in the genital area. 3 of these patients had inguinal lymph node metastases and all 3 patients died within a year of their diagnosis, though only one of these could be directly attributed to EMPD. The mean age at presentation of patients who had invasive disease was 79.9. The mean thickness of the primary tumour which metastasized to regional lymph nodes was 5.83 mm (range: 1.2–9.3 mm), while for cases with no proven lymph node metastasis, the mean thickness of the tumour was 5.11 mm (range: 0.5–15 mm). For the 18 cases of in-situ EMPD, 15 patients were female and the mean age of presentation was 69.3.
Conclusion: From our series, we note that EMPD occurs more commonly in females, but males more frequently develop invasive disease (p <0.01). The thickness of the tumour does not appear to correlate with the presence of lymph node metastasis. However, due to the rarity of the disease and the limited sample size, these findings need to be assessed on larger studies.

P136

Pathological Reporting of Malignant Melanoma in a Non-Specialist Centre: What has Directed Reporting Practice and How has Reporting Compared to Current Guidelines

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Study purpose is to evaluate and compare report content of excisional specimens of malignant melanoma with current guidelines. A 22 year retrospective review of reports for excision specimens of malignant melanoma generated 609 cases reported by 28 Pathologists. Using current best practice guidelines as the comparator, data items were extracted from all reports. Bench texts and published articles were considered the most likely influence to determine report content in the period and concordance with those was evaluated. While publications over the years based different emphasis on data items, Breslow tumour thickness was always most important. Microscopic component of a report often exceeded suggested best practice but was inconsistent and underachieved compared to current dataset guidelines. Clarke's level 1 melanoma was poorly reported. Mitotic index, lymphovascular invasion, satellites, perineural invasion, growth phase, tumour infiltrating lymphocytes and regression were poorly reported but tumour thickness reporting was consistent. All reports used free text and so lacked an aide memoir of reporting items leading to likely absence of negative findings and loss of data items in reports. With free text reporting there is consistency in reporting of data items once endorsed evidence based guidelines become available with best reporting of data items in 2015. Variation in early reporting patterns can be attributed to the current text or guideline influencing the reporting practice. Royal College of Pathologists evidence based reporting guidelines lead to conformity in reporting. There is better reporting of Clarke's level 1 melanoma. Negative data items are included in reports. Proforma reporting further ensures all data items are included. While currently there may be different uses of proformae in signed out pathology reports, mandatory incorporation of the current dataset proforma and updates should be a basic principle of a laboratory information management system.

P137

Perineural Invasion in Cutaneous Squamous Cell Carcinoma: Incidence and Association with Metastasis

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Background: Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer. Most cSCC are treated surgically, however, some cases metastasize. Several studies showed that the presence of high-risk factors including head and neck involvement (HN), size >2 cm, depth of invasion >4 mm, perineural invasion (PNI), lymphovascular invasion (LVI), excision margin <4 mm and poor differentiation, may contribute. Our study looked at the incidence of high-risk factors in our cohort and examined any association with metastasis.
Methods: We reviewed 234 cases of primary cSCC in a single centre from July to December 2016. High-risk factors were recorded and any pathological metastasis to date.
Results: Overall incidence of risk factors in our cohort were HN 70.9%, size >2cm 12.0%, poor differentiation 9.8%, LVI 1.7%, depth >4mm 21.8% and margin <4mm 85.5%. PNI was reported in 8 of 234 cases (3.4%), less than the reported incidence of 5-7%. Five of 234 cases (2.1%) were noted to have a pathologically confirmed metastasis. Of these 5 cases, PNI was found in 2 (40%) while only 2.6% (6/229) of cases without metastasis had PNI ($p = 0.0097$). Other risk factors were not statistically different between the two groups, including HN (100% vs 70.3%), size >2cm (0% vs 16.6%), poor differentiation (40% vs 9.7%), LVI (20% vs 1.4%), depth >4mm (60% vs 25.7%) and margin <4mm (80% vs 87.1%).
Conclusion: In a single centre analysis of 234 cases of cSCC, we showed that PNI appeared to be significantly associated with subsequent metastasis.

P138

Merkel Cell Carcinoma (Primary Cutaneous Neuroendocrine Carcinoma)

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Background: Merkel cell carcinoma (MCC) is an uncommon and aggressive primary cutaneous neuroendocrine carcinoma. It was first described in 1972. Commonly occur in head and neck (HN) of elderly people usually on sun-exposed areas, followed by upper extremity, lower extremity and trunk. The median age of presentation is 66 years and there is slight male predominance (3:2). About 75–83% of patients eventually develop regional nodal and distant metastases. The aim of this study is to compare the MCC cases in a single centre analysis to the cases documented in the literature.
Methods: We reviewed 26 cases of MCC in a single centre in the period from 2011 to 2017. Parameters including age, gender, site, lymphovascular invasion (LVI), unknown primary site and metastasis were checked in comparison to the literature.
Results: All of the cases were over 60 years of age, head and neck was by far the most common site of involvement 54% (N:14), there was a slight male predominance (3:2), unknown primary site was reported in 19.2% (N: 5), lymph node (LN) metastasis was found in 58% (N:15), LVI in 27% (N: 7), extra-nodal metastasis in 31% (N: 8).
Conclusion: In this series of MCC cases; all patients were elderly (>60-year-old). Head and neck was the most common site of involvement (54%). LN metastasis took place in 58% of cases, while extra-nodal metastasis occurred in 31%.

P139

Pyodermatitis-Pyostomatitis Vegetans in childhood

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Pyodermatitis-pyostomatitis vegetans is a rare polymorphous inflammatory disorder of the skin and oral mucosa first described by Hallopeau in 1898. It is characterized by a pustular eruption in the oral mucosa and vegetating plaques involving groin and axillary folds. It's association with inflammatory bowel disease is well known. The diagnosis is made on clinical presentation, histological features, negative direct and indirect immunofluorescence, eosinophilia, and exclusion of infection. A 15 year old girl with ulcerative colitis developed recurrent oral ulceration for one year followed by crusted papules of the axilla and scalp, while her bowel disease was reasonably controlled. Oral and skin biopsies showed epithelial hyperplasia, intraepithelial and subepithelial microabscesses with suprabasal acantholoysis and an inflammatory infiltrate including lymphocytes, neutrophils, eosinophils and histiocytes. The patient had several hospital admissions. She was treated with prednisolone, dapsone and methotrexate and appears to have only responded to a combination of infliximab and methotrexate.

P140

A Long Lasting Case Report of Malignant Lesion: 7 Year History of Nevoid Melanoma of the Skin in a Right Sole for 70 year old Male

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Purpose of the study: To introduce a long lasting case history of nevoid melanoma of the skin and to remind that malignant melanomas which histologically resemble ordinary benign melanocytic nevi remain one of the most difficult diagnoses in dermatopathology. Nevoid melanoma is uncommon, representing approximately 1% of all cutaneous primary invasive melanomas. Nevoid malignant melanoma appears to affect both males and females at any age, although most patients are in their fourth or fifth decade. Nevoid melanoma usually presents as a slowly enlarging papule or nodule involving the trunk, proximal extremities, or less commonly the face.
Methods: In 2003, a 70 year old male was appointed to department of plastic surgery after 2 years of non-healing ulcer in his right sole.
Summary of results: The first diagnose of biopsy-ulcerating melanoma, Clark's level of invasion V. Five times surgical removing of recidives was performed in 2004, 2005, 2007, 2008 and 2009 year. After 3 years of diagnosed nevoid melanoma, patient received chemotherapy with Dacarbazine. In 2004, two enlarged lymph nodes of the groin were removed, neoplasm lesions were not found. In 2007 and 2009 cytology aspiration of enlarged groin lymph nodes were done – atypical polymorphic cells were found and these changes considered as a malignant tumour. BRAF gene mutation not found.
Conclusions: After 7 years of full clinico-pathological exploration, 17 times of hospitalization, increased conglomerate of groin lymhp nodes of 5x5 centimeters was identified during ultrasonography. Doctor's assembly decided to appoint palliative chemotherapy due to unhealing ulcer in right sole, comorbidities and elderly age (77 year). Patient passed away in 2010.

P141

Understanding Angiogenesis in Squamous Cell Carcinoma with Loss of Type VII Collagen

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It is not known why individuals with severe generalized recessive dystrophic epidermolysis bullosa (RDEB), a rare inherited blistering disorder caused by mutations in the COL7A1 gene, develop scarring and aggressive and sometimes fatal squamous cell carcinomas (SCC). Our group previously demonstrated that loss of type VII collagen (Col7) in SCC results in increased angiogenesis in vitro and in vivo. An angiogenesis protein array showed upregulation of Vascular Endothelial Growth Factor (VEGF), in SCC cells with knock-down (KD) of Col7, whilst RDEB SCC patient samples displayed increased VEGF expression compared to non-EB SCC controls. To understand VEGF-mediated angiogenesis in RDEB SCC further, we compared RNAseq data from primary keratinocytes with KD of Col7 to two existing data sets in the literature using several cancer cell lines treated with the anti-VEGF antibody, bevacizumab. We identified some interesting genes in common including the novel putative phosphatase, PALD1. PALD1 encodes Paladin, a recently discovered regulator of angiogenesis. VEGF binds to and activates VEGF Receptor-1 (VEGFR-1) leading to angiogenesis, an essential requirement for tumour growth and metastasis. Stable KD of Col7 was established using shRNA and cells were used in a mouse xenograft model (n=7 per group). Protein expression levels were assessed by Western blot analysis and immunohistochemistry. Increased VEGFR-1 and reduced Paladin expression were observed in shCol7 cells compared to shC cells using Western blot analysis. Moreover, increased VEGFR-1 expression was observed in Col7 KD xenografts compared to xenografts with Col7 present and Col7 KD xenografts with recombinant Col7 (n=7). Our findings demonstrate that VEGFR-1 and potentially Paladin are significant to the increased angiogenesis observed in SCC with loss of Col7 and are further evidence that anti-angiogenic therapies may be beneficial to patients with RDEB SCC.

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P142

Cutaneous Rosai Dorfman Disease: A Case Report

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Rosai Dorfman disease (also known as sinus histiocytosis with massive lymphadenopathy) is a benign and rare entity with an unknown aetiology. Extranodal presentations of the disease are uncommon and can affect anywhere in the body. The skin is thought to be one of the more common extranodal sites affected, but reported cutaneous manifestations of the disease are few in number. It generally occurs in an older population (60 years and older) and with a slight predilection for females. We report a case of a 58 year old female with cutaneous Rosai Dorfman disease who initially presented to her GP with a nodule on her right flank, together with papules and macules on her lower legs that had developed over a period of a few weeks. She was referred to the rapid access dermatology clinic and a punch biopsy was taken. At the time of examination she also provided a history of uveitis and shortness of breath. Sarcoidosis was provided as a possible clinical diagnosis. Microscopically a dense infiltrate of large eosinophilic cells with vacuolated cytoplasm and showing emperipolesis were present. The large cells were found to express CD68 and S100, but were negative for CD1a. The background contained a moderately dense mixed inflammatory infiltrate that was seen extending into the deep dermis and subcutis. The morphological and immunohistochemical features were consistent with a diagnosis of cutaneous Rosai Dorfman disease. Rosai Dorfman disease is usually self-limiting and regarded as being part of a benign process, but cases of chronic disease requiring more aggressive therapies have been reported in the literature. When a histological diagnosis of cutaneous Rosai Dorfman disease is made this should alert the clinician as further investigation of the patient would be necessary to exclude any systemic involvement, and though rare, possible underlying malignancy.

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Development of a Quantification Tool to Estimate the Tumour Cell Percentage in Lung Adenocarcinoma Samples

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Introduction: The current pathologist-dependent visual estimation of the tumour cell percentage (TCP) based on haematoxylin-eosin (HE) tissue slides is an issue when selecting samples for molecular tests that guide targeted therapy. Inaccurate evaluation can lead to unsuitable decisions by inappropriately selecting or discarding a case for subsequent molecular analysis. An objective method for determining the TCP is not yet available for daily practice.
Material and methods: We developed a methodology which consists in performing anti-TTF-1 immunohistochemistry (IHC) on lung adenocarcinoma (ADC) slides followed by whole slide imaging and computer-assisted cell counting. After manual delineation of the tumour area on the slide image, automated segmentation of the TTF-1-positive and negative cells was performed. Two methods were then applied for the TCP quantification: a direct nucleus count and an estimation of the number of cells based on the nucleus surface areas (to compensate for segmentation errors). The TCP was visually estimated by 3 pathologists on 40 lung ADC samples to assess inter- and intra-observer variability. In order to establish a gold standard, we also performed accurate manual counts for 10 cases. We compared the results of the quantification with both the pathologist estimations and the gold standard. Concordance analysis was carried out using Lin's correlation coefficient (rc).
Results: Inter- and intra-pathologist concordance levels for TCP estimation were moderate (rc: 0.56–0.77 and rc: 0.6–0.78, respectively). In contrast, the two quantification methods showed a very high concordance together (rc: 0.97) and also with manual counts (rc > 0.99), demonstrating the high accuracy of our quantification tool.
Conclusion: To eliminate interobserver variability in TCP estimation, we developed a tool combining IHC and image analysis that provides an objective and accurate TCP quantification. This IHC-based approach could be adapted for other cancers.

P146

The Role of Dissection Practitioners in Fresh Tissue Handling For Genomic Medicine

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The 100,000 Genomes Project has the potential to revolutionise the diagnostic process for cancer patients, introducing Whole Genome Sequencing (WGS) with a view to integrating personalised medicine as part of routine cancer diagnostics. WGS requires high quality DNA, obtained by collection of samples from fresh tumour tissue. This has necessitated introduction of new processes for the receipt and handling of unfixed resection and biopsy specimens in the histology laboratory. Initially in our laboratory, tissue sampling for the project was performed by pathologists. However, the increase in patients eligible for the project, coupled with the national shortage of pathologists and increasing overall diagnostic workload, highlighted the need for change. A model was devised to involve scientists in fresh tissue sampling for genomic medicine. Two dissection practitioners were trained to coordinate and perform sampling of the majority of fresh specimens for the 100,000 genomes project, including cancer resection and biopsy specimens. This was found to be a success, impacting positively on the pathologist team by minimising interruption to routine diagnostic work and streamlining the fresh tissue sampling process, improving efficiency. Scientist involvement in fresh tissue handling also provided opportunities for career progression and continuing professional development, in addition to improving dissection practitioner integration into the wider diagnostic team. In conclusion, this project has successfully demonstrated a key role for dissection practitioner involvement in fresh tissue handling for genomic medicine. This may help to facilitate the successful integration of fresh tissue sampling in histology as part of the routine diagnostic pathway, providing much needed support for the pathologist workforce when genomic medicine is commissioned as a mainstream NHS clinical service later this year.

P147

Improved Fixation to Improve DNA Quality from Formalin-Fixed Paraffin Embedded Tissue

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DNA sequencing-based diagnostic techniques are becoming an increasingly important tool in pathology. However, formalin fixation damages DNA through fragmentation and DNA:protein cross-link formation. Consequently, formalin-fixed tissues have poor nucleic acid recovery and/or quality, which presents a problem for sequencing. Although buffering formalin has putative beneficial effects on DNA quality, there have been no attempts to optimise formalin-based tissue fixation protocols. This study investigated the effect of formalin concentration and duration of fixation on DNA quality, morphology and immunohistochemistry. Tissue from 3 organs (uterus, liver, colon; n=8) was fixed in 2.5%, 5%, 7.5%, 10% and 12.5% buffered formalin for 24 or 48h. Tissues were paraffin-embedded and stained with H&E (morphological evaluation), CK7 and SMA (immunohistochemistry). As a measure of functional DNA suitable for next generation sequencing, a qPCR-based approach was used: amplification of a 180 bp FTH1 gene region was compared against standard curves generated from fresh high molecular weight human genomic DNA (peripheral blood). These samples were also compared to DNA extracted from fixed tissue produced by our clinical laboratory using routine, non-buffered saline-based fixation. Reducing formalin concentration to 2.5–7.5% markedly improved amplifiable DNA 1.8–2.5-fold relative to 10% buffered formalin. A comparison with contemporary archival tissue fixed in unbuffered formalin demonstrated that the traditional method resulted in a 74–95% reduction in amplifiable DNA quality. By contrast, increasing fixation time from 24 to 48h had a minimal detrimental effect on DNA quality. Morphology and immunohistochemical staining were unaffected. These findings indicate that optimising tissue fixation by reducing formalin concentration on a background of buffered saline significantly improves the quality of recovered DNA without compromising morphology or immunohistochemical sensitivity.

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Detection of Tumour-Infiltrating Lymphocytes by Content Based Image Retrieval

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Detection of tumour-infiltrating lymphocytes (TILs) is crucial to the study of the tumour microenvironment and to determine the applicability of cancer immunotherapy. Immunohistochemistry (IHC) is the tool of choice, but different methods of assessing TILs abundance and location can be chosen. Semi-quantitative, manual methods are subjective to observer perceptions and difficult to replicate. We have developed an automated quantitative method by using a registration method involving registration of warped Aperio scanned images of sequential sections stained by IHC for CD3 and EGFR (BerEP4 clone) using Strataquest software v.6.0 (TissueGnostics, Vienna). We have developed an automated methods to count lymphocytes in stromal and epithelial areas with minimum computing time (1-2 min per section). Areas occupied by lymphocytes in the epithelium, stroma and total tumour area can also be measured to calculate the density of infiltration and distance of stromal lymphocytes to the epithelium. This method speeds up the laborious IHC assessment and provides pure quantitative data on TILs infiltration.

P149

A Novel Machine-Learning Approach for Segmentation of Tumour Epithelium in Colorectal Cancer

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Purpose of the study: Automated tumour segmentation from histological images is a fundamental aspiration of digital pathology. There are however many artifacts, such as staining variations, which can confound image analysis.
Methods: Here we propose a novel machine-learning approach to the segmentation of the tumour epithelium in histopathology images from colorectal cancer cases, which can avoid the effect of these artifeact. This consists of (a) a set of novel features that encodes meaningful information about the appearance and shape of the region of interest and (b) a novel level set formulation where contour evolution is controlled by a probabilistic model of the appearance of the region.
Results: In order to compare the performance of our approach against the state-of-the-art histology segmentation methods, we adopted Precision, Recall, and F-measure metrics calculated at the pixel level. Results show that our method performs very well on both TMA and WSI and outperformed other competing methods.
Conclusions: We have presented a novel morphometry-based approach to the segmentation of the tumour epithelium in histopathology image, which is i) robust to staining differences; ii) robust to the presence of noise, scanner-dependent intensity inhomogeneities, and laboratory-dependent staining differences; iii) able to cope with a limited number of training samples.

P150

TuPaQ: Tumour Parcellation and Quantification

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Purpose of the study: Tumour identification and quantification in colorectal cancer (CRC) histology images is a fundamental component in the digital pathology toolbox. The aim of this study is to demonstrate the robustness of our automatic image analysis pipeline in identifying and segmenting CRC tumour epithelium regions in images of TMA cores for tumour quantification.
Methods: This study introduces a novel image analysis tool, we called Tumour Parcellation and Quantification (TuPaQ). TuPaQ is a machine-learning approach to identification and segmentation of stromal and tumour epithelial regions for tumour quantification. TuPaQ consists of 3 stages. First, we segment the epithelium using a novel approach based on level set and fuzzy c-means. Second, we distinguish between normal and tumour epithelium using Self Organizing Maps (SOMs) of two feature sets, previously trained on manually labeled images. Finally, we calculate the number of nuclei in tumour epithelial and stromal regions for tumour quantification.
Results: The study was carried out using 286 images of TMA cores derived from CRC tissue samples which were manually annotated to highlight the area of invasive tumour using HALO software. The mean size of tumour area obtained by TuPaQ was very close to the reference value of manual marking. The automated tumour quantification method correlated very strongly with manual estimates, yielding $r = 0.956$ ($p<0.001$).
Conclusions: we present an automatic tumour parcellation and quantification approach for CRC, which is easy-to-use, allow for minimum user intervention, and can efficiently identify tumour epithelial regions and provide the number of nuclei in the tumour epithelial and stromal regions with significant accuracy, sensitivity, and specificity.

P151

An Innovative Method to Change Gene Dosage by CRISPR/Cas9

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Gene dosage can have a major impact on cell biology although it has been difficult to study using in-vitro models. CRISPR/Cas9 is a gene-editing technology which could facilitate gene dosage studies through sequential knockout of gene alleles. We have developed a "gene dosage" model through (i) using Cas9 nuclease mRNA rather than expression plasmids, (ii) using a fluorescently labelled FAM-6 tracr complexed with guide RNA and (iii) using High Resolution Melting (HRM) analysis to screen for mutations. HCT116 cells, wild-type for TP53, were transfected with different molarities of FAM-6 tracr labelled and guide RNA targeting different exons of TP53 and selected by Fluorescence Associated Cell Sorting (FACS). Single-cell colonies were isolated, expanded and tested for mutation in the targeted region by PCR/HRM. Out of 32 clones tested, 12 have shown aberrant melting by HRM, giving a targetting efficiency of 37.5%. One clone was sequenced and a heterozygous mutation found — in this case comprising a single base deletion in exon 3. mRNA sequencing confirmed the mutation was expressed and western blotting for p53 showed the presence of both the wild type and the expected truncated protein bands. Changes in expression of MDM-2 isoforms suggested a functional effect of the induced TP53 mutation. A second-hit on the heterozygous clone produced was attempted by repeating the same process and abnormal melting patterns were again observed, even though sequencing was made diffult by the presence of high GC content and secondary structure of TP53. In conclusion, we have developed an in-vitro model to study TP53 gene dosage effects. The tool is efficient and applicable to any gene. Importantly, we have used Cas9 mRNA and labelled tracr/guideRNA to isolate likely mutated cells and HRM for rapid mutation detection.

P152

The First Successful Implementation of Voice Recognition Software for the Macroscopic Description of Histopathology Specimens in Cut Up – Reducing Costs, Improving Accuracy, Reducing Turnaround Times and Improving Patient Care

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The use of voice recognition (VR) software by consultant pathologists to generate histopathology reports is well established. However the pathology cut-up room is a fairly noisy and harsh working environment not ideally suited to the use of voice recognition software. The traditional way to record the macroscopic description of pathology specimens is via digital recording or Assistant Technical officers (ATO's) scribing which then has to be typed by secretarial staff into the Laboratory Information Management System (LIMS). We demonstrate the first successful introduction VR in histopathology cut-up.
Benefits:
– Canned text options allows quick and accurate description of simple cases
– Free text can be used for the accurate description of complex cases
– Electronic generation of workload figures
– BMS staff are able to dissect and describe specimens singlehandedly
– The use of VR in cut-up saves circa 7.75 man-hours / day
– Secretarial time was saved- our department was able to give up one secretarial post (via natural wastage). This money was reinvested in technical staff in the laboratory.
– Turnaround times are improved
Summary: The use of VR technology in the histopathology cut-up environment is feasible and achievable. It improves accuracy and turnaround times and results in cost reductions which can be invested elsewhere in the laboratory. Ultimately this leads to improved morale of staff and improved clinical care.

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