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Pathological Society
Understanding Disease — Guiding Therapy

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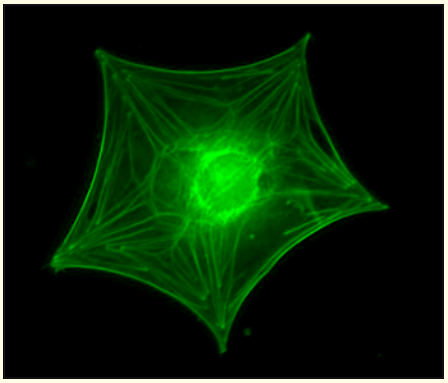
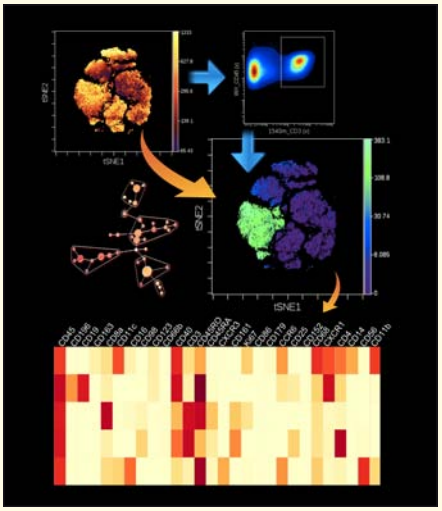
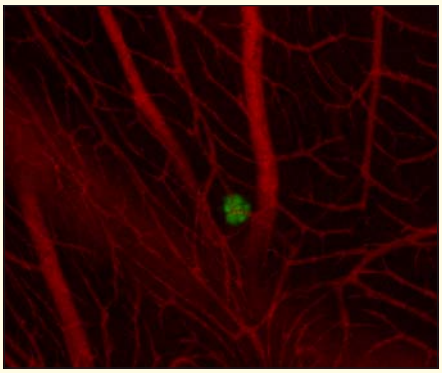
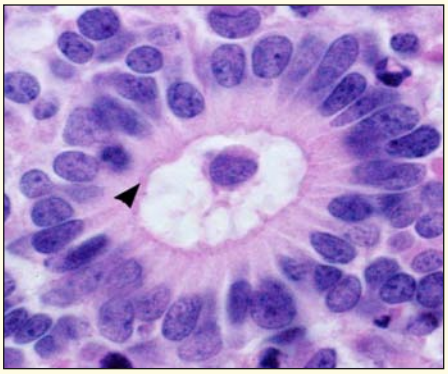
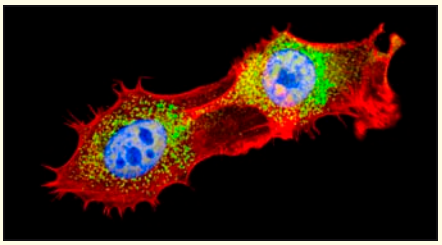

Abstracts

WINTER MEETING

21 – 22 January 2020

Molecular and Digital Pathology: The Future

3rd Joint Meeting with the Royal Society of Medicine
212th Scientific Meeting of the Pathological Society of Great Britain & Ireland

Hosted by
The University of Edinburgh and
The University of Manchester

Venue
The Royal Society of Medicine,
1 Wimpole Street, Marylebone,
London W1G 0AE

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KEY TO SYMBOLS

Ⓟ = Presenter

*** = Supported by a grant from the Pathological Society of Great Britain & Ireland**

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PHOTOGRAPHS

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(top centre and top right): Dr Carlos de Figueiredo, a post-doctoral researcher in Melanoma at LOORG (www.loorg.org), University of Liverpool.
(lower left): Dr Anne Herrmann, a post-doctoral researcher at the University of Liverpool, investigating GFP-labelled neuroblastoma cells in chick embryo model.
(lower right): Dr Mateus Milani, a post-doctoral researcher at the University of Liverpool, working on proteins involved in mitochondrial fission.

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Invited Speaker Abstracts

S1

QuPath and Image Analysis in Pathology

Ⓟ P Bankhead

University of Edinburgh, Edinburgh, UK

Computational analysis of whole slide images has huge potential to impact the field of pathology, helping pathologists assess samples more rapidly, quantitatively and reproducibly. However, the diversity of pathology data and subtlety of analyses make it extremely challenging to move computational algorithms beyond the 'proof-of-concept' stage into becoming broadly applicable. Myriad potential sources of variation, error and bias make it necessary to collaborate closely across disciplines to first develop, then understand, and finally validate analysis algorithms that can be applied reliably. QuPath is open source software designed specifically for whole slide analysis and digital pathology (https://qupath.github.io). A key motivation behind its creation is to provide a user-friendly platform that enables pathologists, biologists, image analysts and machine learning experts to work together to maximize the insights we can extract from medical images. Because it is freely-available, flexible and extensible, QuPath can be used to quickly develop and share new analysis methods without cost. This talk describes the background to QuPath, its current applications and latest developments, and how its adoption by researchers worldwide enables and accelerates biomedical research and advances in digital pathology through open science.

S2

Intelligent Software Solutions Support and Enable Pathologists to Guide Clinically Relevant Decisions

SE Coupland[1]; Ⓟ R Huss[2]

[1]University of Liverpool, Liverpool, UK; [2]Pathology Consultant, Institut für Pathologie, Universitätsklinikum Augsburg, Germany

The tasks for pathologists have become increasingly demanding and will be even more challenging in the digital future. The role of the pathologist has evolved from exclusively describing the phenomenology of a disease to a therapy gatekeeper based on identification and management of complex information that is available in a piece of tissue. It is no longer adequate to classify a cancer type based on the mere morphology but also rather accurately measure the quantity and dimensions of all different components in the tissue specimen and immediately link the morphology to the patient's genome and other meta-data that become available. Standardized software allows pathologists not only to manage all available information but also generate more actionable knowledge when applying machine or deep learning solutions. While machine learning assists the pathologists to do what a pathologist does, only faster and more accurate (while counting mitotic figures still seem to be a challenge), deep learning tools can generate novel insights depending on the quantity and quality of available data. In both instances, it is the human factor pathologist to validated and assess any software-based solutions regarding diagnostic plausibility and clinical utility based on experience and empirical knowledge. Even without the sophistication of artificial intelligence, different software solutions can assist the pathologists in identifying regions-of-interests for further thorough exploitation, document the complete scrutiny of a digitized image as part of a certified quality measure and understand tumour heterogeneity in its entirety. Especially in the dawning age of immunotherapies and complex combination treatments along with cancer-specific scoring algorithms for different antibodies on various staining platforms and concordant molecular parameters, validated software solutions guide the decisions by the pathologist without depriving him of the role as the ultimate diagnostic authority.

S3

Optical Imaging: Beyond the Microscope

Ⓟ CS Herrington

University of Edinburgh, Edinburgh, UK

Light microscopy allows the interrogation of cells and tissues by passing white light through individual cells or thin sections stained to provide contrast. By exploiting other properties of light, optical imaging technologies can generate spatial (including 3D) and molecular information from tissues that can both complement light microscopy and provide images from cells and tissues *in vivo*. Examples include optical coherence tomography, which is already used extensively in ophthalmology, Raman spectroscopy and light sheet microscopy. Advances in laser technology and beam shaping, and in computing science and engineering, are bringing these approaches closer to wider clinical application. Methods that involve cell and tissue labelling hold promise for specific applications, including theranostics in which imaging and therapy are combined, whereas label-free methods are applicable more widely, particularly for the generation of structural information. Ultimately, optical imaging holds promise for the study and investigation of disease, not only in tissues that cannot be biopsied, such as the retina and the coronary arteries, but also more widely in the clinical investigation of patients.

S4

Role Modelling in Medical Education

Ⓟ V Passi

King's College London, London, UK

This presentation will highlight the importance of role modelling in medical education. Excellence in doctor role modelling involves demonstration of high standards of clinical competence, excellence in teaching skills and humanistic personal qualities. Positive role models not only help to shape the professional development of our future doctors but they also influence their career choices. The presentation will explore the characteristics of effective role models, the impact of role models and methods of enhancing positive doctor role modelling in medical education.

Ⓟ = Presenter ✱ = Supported by a Pathological Society Grant

S5

Why I Want to be a Pathologist

Ⓟ AFI Matkowski[1]; Ⓟ S Seth[2]

[1]*Manchester University NHS Foundation Trust, Manchester, UK;* [2]*Queen Elizabeth University Hospital, Glasgow, UK*

Pathology is rapidly evolving and offers a variety of subspecialties that could appeal to many medical students. However, the day-to-day life of a pathologist remains shrouded in mystery. Despite being an integral part of clinical medicine, few students consider pathology as a specialty. Role models are key factor in determining career choices but with limited opportunities for pathologists to engage with students, how do we break down stereotypes and encourage students to explore a career in pathology?
Two medical students discuss their experiences of pathology and what has inspired them to pursue a career in this area.

S6

Autophagy and Neurodegeneration

Ⓟ DC Rubinsztein

Cambridge Institute for Medical Research, UK Dementia Research Institute, University of Cambridge, Cambridge, UK

Intracellular protein aggregation is a feature of many late-onset neurodegenerative diseases, including Parkinson's disease, tauopathies, and polyglutamine expansion diseases (like Huntington's disease (HD)). Many of these mutant proteins, like that causing HD, cause disease via toxic gain-of-function mechanisms. Therefore, the factors regulating their clearance are crucial for understanding disease pathogenesis and for developing rational therapeutic strategies. We showed that the autophagy inducer, rapamycin, reduced the levels of mutant huntingtin and attenuated its toxicity in cells, and in Drosophila, zebrafish and mouse HD models. We have extended the range of intracellular proteinopathy substrates that are cleared by autophagy to other related neurodegenerative disease targets, like alpha-synuclein in Parkinson's disease and tau in various dementias. While autophagy induction is protective in models of various neurodegenerative diseases, many of these diseases are associated with compromised autophagy. I will discuss two of our studies describing how autophagy can be regulated. The first will consider how the amino acid leucine regulates autophagy via mTORC1. The second will describe our drug repurposing efforts to identify compounds already used in humans for other indications that may be suitable as autophagy inducers in the brain.

S7

The Challenges of Interpreting Genetic Variants

Ⓟ IM Frayling

Cardiff University, Cardiff, UK

The pathology of cancer is a function of genetic variants, a combination of inherited constitutional variants and acquired somatic variants. All genetic variants arise as a result of mutations, which in the case of constitutional variants occur in the germline. As genetic testing of individuals and tumours becomes more prevalent so more variants are being found and their interpretation is a major challenge. Whether a variant is pathogenic may be of critical importance in the care of a patient and a whole new science is growing up to address this, using multiple lines of evidence, often including the results of standard and molecular pathological as well as genetic tests. The qualitative American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG/AMP) variant interpretation guidelines, as part of the US ClinGen/ClinVar initiative https://www.ncbi.nlm.nih.gov/clinvar/ are becoming widespread in their application, but were actually predated by a quantitative system based on Bayesian statistics introduced by the International Society for Gastrointestinal Hereditary Tumours (InSiGHT). https://www.insight-group.org/criteria/ As ClinGen recognises Variant Curation Expert Panels (VCEP), so the InSiGHT Variant Interpretation Committee (for DNA mismatch repair (MMR) genes) is now the FDA-recognised VCEP for MMR genes. https://www.ncbi.nlm.nih.gov/clinvar/submitters/500189/ This is necessitating mapping of the InSiGHT to ACMG/AMP criteria, so that variant interpretation is not disparate with concomitant risks to patient safety. And, while entries on ClinVar as a whole are not curated, all variants that come under the responsibility of a VCEP are curated, and so quality of interpretation is assured. A corollary of this is that data relevant to variant interpretation must be publicly available for inspection, but this may conflict with commercial and proprietary, as well as ethical, legal and social considerations in some jurisdictions.

S8

Molecular Genetics of Colorectal Cancer

Ⓟ I Tomlinson

Edinburgh Cancer Research Centre, Edinburgh, UK

In this talk, I present an overview of the genetic changes that occur in colorectal cancer (CRC), and their importance for tumorigenesis and for the outcomes of patients whose tumours follow particular molecular pathways. I show that most CRC driver mutations occur at the pre-malignant stage, and that malignancy is characterised by copy number changes and genome doubling. I highlight the interesting subset of cancers that have acquired defects in DNA repair and the specific genetic pathways into which these cancers appear to be led by their genomic instability. Throughout the presentation, I will draw heavily on the work of the CRC domain in the 100,000 Genomes Project and will present recent findings from that effort.

S9

The Genomics of Dermatological Cancer

Ⓟ D Adams

Wellcome Sanger Institute, Hinxton, UK

Virtually no other tumour type is associated with so many different forms as skin cancer. Histologically, skin tumours may arise from epithelium, including epidermis, hair follicle, sebaceous or sweat gland, melanocytes, dermal-associated mesenchymal structures or tissue resident immune cells, making for a diversity of clinical presentations. Although often treatable surgically, some skin tumours are associated with significant morbidity, or in the case of tumour types such as angiosarcoma, Merkel cell carcinoma and some sarcomatoid/spindle cell carcinomas, an extremely poor prognosis.
There are currently several major barriers to progress in the field of molecular skin cancer pathology. Firstly, many skin tumour subtypes have never undergone molecular profiling, or if they have, targeted sequencing has been used and the number of cases analyzed has been so limited that firm conclusions about the profile of driver genes, DNA mutational signatures and germline alleles has not been possible. Virtually no studies have explored these tumours' epigenome. Secondly, since many skin tumours are rare no one pathologist has sufficient cases to draw statistically robust conclusions meaning that a team science approach is required. Finally, until now, the technology to analyze cases from FFPE material has been a major hurdle to progress. I will discuss recent advances in molecular skin pathology and provide a future vision for the genomic analysis of these conditions.

S10

Targeting Regulated Cell Death in Disease: Apoptosis and Beyond

Ⓟ SW Tait

Cancer Research UK Beatson Institute, Glasgow, UK

Aberrant levels of cell death, either too much or too little, has key roles in diseases ranging from cancer to autoimmunity. While apoptosis is the best understood form of cell death, it has recently become apparent that regulated cell death comes in many different flavours. Other major types of cell death include pyroptosis, necroptosis and ferroptosis. How a cell dies can have massively different effects on its environment - for instance pryoptosis is an inflammatory type of cell death whereas apoptosis is considered non-inflammatory. During my talk, I will discuss different forms of cell death, how they can be detected and how they contribute to disease. Emphasising the importance of how a cell dies, I will discuss our recent data showing that modulating the mode of cancer cell death can have a profound impact on tumour growth. Excitingly, this opens new avenues for therapeutic exploitation.

S11

Molecular Pathology of Breast Cancer

Ⓟ E Provenzano

Addenbrookes Hospital, Cambridge, UK

Traditional morphological classification of breast cancer based on histological type and tumour grade, along with ER and HER2 status as determined by immunohistochemistry and ISH, remain the cornerstones for clinical management of breast cancer. Advances in our understanding of the molecular biology of breast cancer have changed the way we think about breast cancer classification. Gene expression profiling studies have identified four intrinsic subtypes of breast cancer largely distinguished by oestrogen receptor (ER) and HER2 gene expression, however attempts to define immunohistochemical surrogates of these subtypes show moderate concordance at best limiting their transfer into routine practice. There is still considerable genetic heterogeneity within these subtypes, and triple negative breast cancers can be further subdivided into at least 4 groups. Commercial genomic tests based on gene expression signatures as predictors of risk are now widely used in adjuvant therapy decision making for ER positive, HER2 negative disease. Breast cancer is largely driven by copy number alterations, with the majority of individual gene mutations occurring at low frequency. Future advances include use of whole genome sequencing to identify actionable mutations and genetic signatures allowing more targeted therapeutic interventions with the ultimate goal of personalised cancer therapy.

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Plenary Oral Abstracts

PL1 *

Ethanol Induction of Colorectal Tumours and Precursors in a Mouse Model of Lynch Syndrome

Ⓟ G Cerretelli; Y Zhou; MJ Arends

University of Edinburgh, Division of Pathology, Institute of Genetics and Molecular Medicine, Edinburgh, UK

Lynch Syndrome (LS) confers inherited cancer predisposition due to germline mutations in one of the DNA mismatch repair (MMR) genes. MMR is a DNA-damage repair pathway involved in the removal of base mismatches and insertion/deletion loops caused by several endogenous and exogenous factors. Loss of MMR through somatic alteration of the wild-type allele in LS results in defective MMR (dMMR). Ethanol and its metabolite acetaldehyde, are classified as group one carcinogens by the IARC. Aldehydes are very reactive molecules that constitute a serious threat to cellular integrity by causing a range of DNA lesions. However, DNA repair pathways responsible for correcting such lesions remains unknown. We hypothesized that MMR plays a role in protecting the cell from ethanol/acetaldehyde induced DNA damage. In this study, we aim to determine if there is a gene-environment interaction between dMMR and ethanol/acetaldehyde that accelerates colorectal tumourigenesis. We used a conditional Msh2 knockout mouse model that mimics the LS patients' pattern of MMR gene inactivation. The LS model mice (6-8 weeks of age) were fed either with 20% ethanol in drinking water or normal drinking water. Most of the ethanol-treated mice demonstrated large intestinal hyperproliferation, adenoma formation and, in some cases, invasive adenocarcinoma within 6 months (11/15), compared with one case of intestinal tumour formation after 15 months in the water-treated mice (1/15). The quantification of the dMMR crypts in LS mouse colon has shown an increased number of dMMR foci in ethanol-treated mice compared with the control group. Preliminary results indicate that long-term ethanol treatment induced acceleration of dMMR-driven large intestinal tumour formation. Possible mechanisms may include increased DNA damage/mutation rate and selection by avoidance of apoptosis that leads to an acceleration in intestinal tumour development.

* ***Supported by the Pathological Society of Great Britain & Ireland PhD Grant.***

PL2 *

KLF2 is a Global Regulator of NOTCH2 and NF-κB and Mutation Abolishes its Repressor Activities

Ⓟ MA Rust; M Wang; J Gao; F Cucco; A Clipson; Z Chen; MQ Du

University of Cambridge, Cambridge, UK

Splenic marginal zone lymphoma (SMZL) originates from the splenic marginal zone B-cells. It harbours recurrent somatic mutations in KLF2, NOTCH2, NF-κB, BCR and TLR signalling pathways which are essential for the biology of marginal zone B-cells, with deleterious KLF2 mutations being the most frequent. Our previous studies show that KLF2 negatively regulates NF-κB activation by both canonical and non-canonical pathways, and mutation abolishes its ability in NF-κB repression. To expand this observation, we tested whether KLF2 also regulates NOTCH2 activation, and if so whether this is affected by mutation. As expected, wild type KLF2 repressed CSL transcriptional activity triggered by the active intracellular domain of NOTCH2 (N2ICD). Conversely, all 3 C-terminal truncated mutants and 8 of the 10 missense/inframe deletion mutants tested lacked the ability of N2ICD/CSL repression. Confocal imaging analyses revealed remarkable differences in subcellular localisation between wild type and mutant KLF2. Wild type KLF2 showed a diffuse nuclear expression. In contrast, the truncated mutants showed predominant cytoplasmic expression, and 6 of the 10 missense/inframe deletion mutants displayed a "speckled" pattern of nuclear expression. Further analysis confirmed co-localisation between KLF2-H296Y mutant and SC35 (SRSF2), a component of the spliceosome, suggesting that KLF2 may also involve in RNA processing activities. Taken together, our results indicate that KLF2 is a global negative regulator of both NOTCH2 and NF-κB pathways, and mutation inactivates its repressor activities through perturbation of its nuclear localisation, resulting in enhanced NOTCH and NF-κB activities and consequently promoting lymphomagenesis.

* ***This work was made possible by the kind support of the Pathological Society of Great Britain & Ireland PhD Studentship Grant.***

PL3

XRCC1 is a Predictor of an Aggressive Phenotype in Pre-Invasive Ductal Carcinoma In Situ (DCIS) and Invasive Breast Cancer (IBC)

Ⓟ A Al-Kawaz; R Ali; M Toss; I Miligy; K Mesquita; A Green; E Rakha; S Madhusudan

University of Nottingham and City Hospital, Nottingham, UK

Introduction: XRCC1 is a key player in DNA repair particularly base excision repair (BER) and single-strand breaks repair (SSBR). XRCC1 interacts with PARP1 and inhibition of PARP1 in XRCC1 deficient tumours can be an attractive synthetic lethality approach. XRCC1 deficiency delays SSBR which if persists eventually leads to double-strand breaks (DSBs). XRCC1 deficiency/mutations are associated with PARP1 hyperactivity. Our hypothesis is that XRCC1 downregulation is an early event in breast cancer pathogenesis.
Methods: Pure DCIS (n=779), mixed DCIS/IBC (n=239) and IBC (n=1011) were assessed for XRCC1 and PARP1 using tissue microarrays and immunohistochemistry. Protein expression was correlated with clinicopathological parameters and patient outcome. XRCC1 knock out by CRISPR/Cas-9 systems were generated in DCIS cell lines (MCF10DCIS) and triple-negative breast cancer cell line. PARP1 inhibitor Olaparib was assessed for synthetic lethality in XRCC1 deficient cells. Cell proliferation, invasion and migration assays were used to assess the consequences of XRCC1 loss.
Results: XRCC1 deficiency in pre-invasive breast cancer was associated significantly with aggressive tumour behaviour and is linked to increased risk of local recurrence. Tumours with low XRCC1/high PARP1 demonstrated aggressive behaviour and were linked to poor outcome in IBC. In vitro, XRCC1 downregulation impairs DNA damage repair. Targeting PARP1 is an exciting approach for synthetic lethality and chemoprevention in XRCC1-deficient breast cancers, including preinvasive DCIS. Our findings show that loss of XRCC1, which was associated with DCIS, could be exploited by PARP1, suggesting a promising therapeutic and chemoprevention strategy in XRCC1-deficient cancer cells.

PL4

This abstract has been withdrawn

Poster Abstracts

P1

An Audit of Toxicological Findings in Coronial Post Mortems

Ⓟ A Borbora[1]; J Robinson[2]; M Cieka[2]; CP Johnson[2]; J Medcalf[2]

[1]Countess of Chester Hospital, Chester, UK; [2]Royal Liverpool University Hospital, Liverpool, UK

Purpose: To examine the usefulness of toxicological examination in routine coronial autopsy examinations performed by our regional forensic pathology unit.
Methods: A retrospective review of all (234) cases undertaken between May and December 2016 was undertaken. The following was recorded: deceased's demographics, place of death, reason for toxicological analysis, presence or absence of toxicological findings, fatal or contributory intoxication, presence of non-prescribed drugs, cause of death, urine and blood alcohol levels, opiate levels, and fatal drug levels.
Results: 71% of the deceased were male. The average age was 56.2 years (male 54.6, female 60.1), and 91% of deaths took place in the community. Toxicological investigations were performed in 63% of cases; the most common reasons being suspected self-harm, past psychiatric history, and history of alcohol or drug misuse. 72% of cases having toxicology were male, with an average age of 51 years (male 48.5, female 56.7). 89% of analyses were positive, 71% of which revealed illicit or non-prescribed drugs or alcohol. 27% died from fatal intoxication. Fatal intoxications were most common in those with previous history of overdose (67%) and drug use (57%). Non-fatal intoxications were commonly seen in hangings (75%), decomposed (74%), and alcoholics (72%). The most frequent fatal drugs were opiates (40%), mixed drugs (24%), cocaine (6%), and anti-depressants (6%).
Conclusions: Fatal intoxications were more common in this series (17% all deaths) than international comparisons. The unit took more toxicology than the all-England average (16%), but the indications for doing so are robust as supported by the low negative rate (11%). It is likely that the low sampling rate means toxicological deaths are being missed. Certainly toxicology should be taken in those with previous drug use or overdose attempts. Further work will include expanding the dataset to cover further years and newer substances.

P2

Infantile Bruising to the Head in Deliberate Upper Airway Obstruction: The Value of Experimental Modelling to the Autopsy

Ⓟ F Green[1]; G Johnson[2]; CP Johnson[3]

[1]Whittington Health NHS Trust, London, UK; [2]Aintree University Hospital NHS Trust, Liverpool, UK; [3]Dept of Forensic Pathology, Royal Liverpool University Hospital, Liverpool, UK

Distinguishing between accidental and non-accidental injury at the paediatric autopsy poses a number of difficulties for the pathologist. With bruising being the most common presenting finding in infants who have been abused, we aimed to characterise whether there were any bruising patterns that might suggest intentional upper airway obstruction. We recruited 31 volunteers and asked them to undertake two trials on an infant resuscitation dummy. In trial 1, they were instructed to force the dummy face-down into a pillow and then in trial 2, to use one or both hands to obstruct the external airways with the dummy face-up. We applied chalk to their fingertips, allowing us to identify the location of theoretical bruise marks that would have been left behind on the infant's head. The chalk marks were allocated to different anatomical zones and the percentage distribution for each zone was calculated and heat maps made to highlight commonly seen patterns. In trial 1, over 80% of thumb and fingertip marks were unsurprisingly in the occipital region of the infant's head. Yet, over 15% of all thumb marks were left on infant's ears and over 10% of fingertip marks were found on the anterior aspect of the face. The most likely zone to find both thumb and fingertip bruising in trial 2 was the frontal region, however, marks were also found occipitally, on ears and periorally. Additionally, vertical or slightly curved runs of finger marks on one side of the face with a contralateral or central thumb mark was a commonly seen configuration.
This work has demonstrated that digit marks can be left anywhere on the head in experimental airway obstruction in infant models, with a large variation in distribution based on the method used. As such, the identification of one or more fingertip type bruises anywhere on an infant's face or scalp should prompt close examination for other markers of upper airway obstruction including petechial haemorrhages and abrasions to the lining of the lips.

P3

This abstract has been withdrawn

P4

Adenomyoepithelioma: A Case Report of a Rare Tumour Diagnosed on Core Biopsy

Ⓟ GG Gupta; SDP Di Palma; MW Warren; CT Taylor

The Royal Surrey County Hospital, Guildford, UK

Introduction: Adenomyoepithelioma (AME) of the breast is an uncommon tumour characterized by dual differentiation into luminal and myoepithelial cells. A spectrum of histological patterns is demonstrated sometimes within the same tumor. Due to their heterogeneity they are a diagnostic challenge especially on a core needle biopsy. Their differentials vary from tubular adenoma to carcinoma. Most cases have a benign course but local recurrences, malignant transformations and distant metastases do occur. Their treatment thus is a complete excision to lower the risk of recurrence and metastasis, which is dependent on a correct diagnosis.
Case Report: A 63 year old woman presented with a non-tender palpable 8 mm lump in her left breast located in the upper inner quadrant. Radiology demonstrated multiple well circumscribed round, lobulated, dense masses. No malignant micro-calcifications were present. It was graded as a P2, M3 and U3 lesion and queried as a fibroadenoma. The core biopsy showed breast acinar structures with epithelial and hyperplastic myoepithelial cells. A provisional diagnosis of a tubular adenoma was made and immunohistochemistry with basal /myoepithelial markers was performed. This showed myoepithelial cells surrounding the tubules and infiltrating the stroma with central areas with supervening necrosis. A diagnosis of AME was made after corroborating the radiological findings with the histological findings on the core biopsy. A complete excision was advised.
Conclusion: AME is an unusual breast neoplasm that can be differentiated from other conditions and accurately diagnosed by a combination of radiological and core biopsy findings to ensure proper management and follow up.

P5

Distribution of Ki67 Expression, Macrophages and pSTAT5 Expression, During Ruminant Mammary Postnatal Terminal Ductal Lobular Unit Development: Insights for Understanding Postnatal Breast Development

D Nagy[1]; CMC Gillis[1]; AL Fowden[2]; J Wills[1]; Ⓟ K Hughes[1]

[1]Dept of Veterinary Medicine, University of Cambridge, Cambridge, UK; [2]Dept of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK

Study purpose: The breast undergoes a striking degree of postnatal development, characterised by periods of isometric and allometric growth leading to formation of distinct terminal ductal lobular units (TDLUs). The ruminant mammary gland also exhibits TDLUs. This study interrogated critical aspects of TDLU development.
Methods: Ki67, Iba-1, and pSTAT5 expression were analysed in the mammary gland of lambs of various ages by immunohistochemistry. Analysis of marker expression, and distribution of macrophages, were by manual quantification and machine learning.
Summary of results: Periods of allometric and isometric growth in the lamb mammary gland are less well-defined than previously suggested, although there is a trend to higher levels of Ki67 expression in neonatal animals compared to pubertal animals. There is a striking variation in Ki67 expression between peripheral and central areas of the developing ductal tree, with peripheral ducts exhibiting higher levels of Ki67 expression as they penetrate the surrounding fat pad. Given the importance of macrophages during postnatal development of the rodent mammary gland, we assessed presence of macrophages (Iba-1 immunohistochemistry). Interestingly, macrophages are significantly more abundant in the central compared to peripheral stroma, whilst numbers of intraepithelial macrophages do not vary between locations. Finally we have demonstrated that STAT5, more typically associated with the pregnant and lactating mammary gland, is phosphorylated in a subset of mammary epithelial cells and immune cells during postnatal development.
Conclusions: Given their distribution, we suggest that stromal macrophages may have functions other than coordination of ductal development, such as immune surveillance around the main ducts. Studying ruminant TDLU development provides new insights into postnatal mammary development and constitutes a valuable model system.

P6

Mammography-Histology-Linking-Model: Breast Cancer Modelling to Predict Histology Using Mammograms

Ⓟ A Hamidinekoo[1]; K Honnor[2]; E Denton[2]; R Zwiggelaar[1]

[1]Aberystwyth University, Aberystwyth, UK; [2]Norfolk and Norwich University Hospital, Norwich, UK

Introduction: Triple assessment is the gold standard for clinically evaluating breast lesions. Patients recalled from mammographic screening, or presenting symptomatically, often progress to biopsy. A significant proportion of these women have benign disease, causing unnecessary morbidity and healthcare costs. We proposed the Mammography-Histology-Linking-Model ($ML_{M<->H}$), to develop a mapping of features between mammographic abnormalities and their histological representation. This is a type of deep learning based computer aided diagnosis system. Publicly available mammograms and histological images were used to develop $ML_{M<->H}$, which was tested on our clinically collected dataset from 103 patients.
Results: We calculated mammographic mass lesion detection and classification (benign or malignant) scores, and the accuracy of the retrieved most likely histological image. The detection model was able to localise mass abnormalities with the Dice score of 0.33 and F-score of 0.33±0.30. Comparing subject-based classification performance using the detected suspicious regions vs. annotated lesions by an expert radiologist illustrated that the trained classification model could classify pre-detected mass including regions with the accuracy of 78.64%, which was 15.54% better than classifying automatically detected regions. The linking model was able to retrieve similar samples with the Image Retrieval accuracy of 71.26%.
Conclusion: Proposed clinical utility of this model includes assisting radiologist classification of indeterminate lesions, and contributing to Multidisciplinary Team Meeting discussion, eg. influencing the requirement for further biopsy or excision. Aside from clinical benefit, the model could also be valuable in education and research.

P7

A Case Report of Recurrence of Adenomyoepithelioma with Metastasis 3 Years Post Initial Surgical Resection

Ⓟ DJB McMahon; O Mikulich; H Gyorffy; K Dillon; G O'Dowd

Letterkenny University Hospital, Letterkenny, Ireland

A 53-year-old Lady presented to clinic with a dry cough for 2–3 months. She displayed no other symptoms. She was noted to have a distant recurrence of malignant adenomyoepithelioma, the primary of which originated in the left breast and was surgically resected 3 years prior to this presentation, with an axillary clearance, and staged at T2N0M0. Metastases were noted in the lungs with bilateral presumed malignant pleural effusions. Biopsy showed mixed epithelial and myoepithelial neoplasia and she was diagnosed with malignant adenomyoepithelioma with a biphasic appearance. Her original biopsy demonstrated epithelial cells surrounded with further atypical epithelial proliferation. Myoepithelial cells were also noted involving focally spindled cell appearances, in keeping with malignant adenomyoepithelioma. Adenomyoepithelioma of the breast is an uncommon tumour characterized by dual differentiation into luminal cells and myoepithelial cells. Although most tumours have a benign clinical course, local recurrences, malignant transformations, and distant metastases have been reported rarely. Given the small numbers involved, and generally benign disease course, it is unlikely adjunctive chemo/radiotherapy or immunotherapy will be recommended in the near future, but the potential for recurrence and metastases necessitates the need for long-term follow up. This case adds to the body of evidence for this approach.

P8

Outcome of Repeat HER2 Testing After Initial Equivocal HER2 FISH Results Using 2013 ASCO/CAP Guidelines and Implications for Impact of Updated 2018 ASCO/CAP in Similar Cases

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Background: Using 2013 ASCO/CAP HER2 guidelines breast carcinomas were considered FISH equivocal if HER2/CEP17 ratio was < 2 and HER2 copy number was >/= 4 and < 6. Repeat HER2 testing was recommended on excision specimen after a HER2 FISH equivocal result on biopsy. The 2013 guideline was used in our Dept from 2015 to 2018. The 2018 ASCO/CAP guideline superseded the 2013 guideline.The majority of 2013 FISH equivocal cases are re-classified as HER2 "negative (with a comment)" with these criteria. The 2018 guideline indicates that a repeat test "may" (rather than "must") be performed on the excision in "negative (with a comment)" cases. Our study aimed to (1) identify HER2 FISH equivocal (2013) breast carcinoma biopsies reported from 2015-2018 in our institution; (2) determine the impact of repeat testing on final HER2 status when a resection was available; (3) identify how these cases would be classified using 2018 guidelines; (4) use this data to inform a decision on repeat HER2 testing on excision in cases "negative (with a comment)" on biopsy.
Methods: HER2 FISH equivocal cases from 2015-2018 (ASCO/CAP 2013 guidelines), and information about repeat testing were identified from the Laboratory Information System.
Results: 50 HER2 FISH equivocal (2013 ASCO/CAP) biopsies were identified.HER2 testing was repeated in 86% of patients who a subsequent excision (30/35). Repeat HER2 testing on the resection clarified HER2 status in 63% of cases - 14/30 negative, 5/30 positive. 37% of cases remained equivocal, all of which would be classified as negative (with a comment) using 2018 guidelines.
Conclusion: 17% (5/30) cases in our cohort were HER2 positive on repeat testing, using both criteria. Due to significant impact of HER2 positivity on therapeutic management, we will continue to repeat HER2 studies in all cases classified as 2018 HER2 "negative (with a comment)", i.e. 2013 equivocal.

P9

Intimal Sarcoma: A Case Series

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Intimal sarcomas are very rare malignant mesenchymal tumours arising in large blood vessels of the systemic and pulmonary circulation. These tumours have an insidious onset, presenting with non-specific symptoms, advanced disease and have poor prognosis.
Case one: 78 year old presented with a four day history of pleuritic chest pain, cough, and haemoptysis. Imaging revealed a large filling defect within the pulmonary trunk. Fine needle aspirate cytology during pulmonary angiogram of the mass was diagnostic.
Case two: 72 year old male presented with progressive shortness of breath, cough and weight loss. Imaging revealed a large tumour involving both pulmonary arteries, almost complete occlusion of the left. Surgical resection confirmed a tumour arising within the pulmonary artery with luminal occlusion and invading the lung parenchyma
Case three: 45 year old woman presented with right homonymous hemianopia and left sided weakness and neglect. Imaging revealed extensive systemic emboli to the kidneys, spleen, and brain arising from a 5cm lesion within the left atrium extending into the left ventricle and the perihilar veins. Surgical resection confirmed a tumour within the left atrium and a branch of the pulmonary vein, infiltrating the lung parenchyma and encasing the pulmonary artery.
Morphologically, all cases showed a pleomorphic spindle cell tumour with scattered admixed highly atypical calls and with extensive necrosis/fibrosis and occasional mitoses. Vimentin was consistently positive in all cases. A diagnosis of intimal sarcoma was made for each. Intimal sarcoma is a rare malignancy, often presenting with insidious symptoms. Precise diagnosis relies on careful evaluation of the surgical specimen, particularly with its respect to it's origin from the vessel wall, as well as correlation with corresponding clinical and radiological data.

P10

A Study into the use of EBUS and the Rate of False Negatives in Diagnosing Malignancy

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Endobronchial Ultrasound (EBUS) is a highly effective procedure commonly used in respiratory medicine to diagnose lung cancer, infections and other diseases causing enlarged lymph nodes in the chest. EBUS enables tissue or fluid samples to be taken by transbronchial needle aspiration (TBNA) for histological analysis. The respiratory unit at our hospital began performing EBUS procedures on patients in 2016 and has expanded its service in subsequent years. This study aimed to quantify the use of EBUS at the hospital from 2016-18 and analyse its effectiveness in detecting malignancy as the team refined their technique.
We retrospectively analysed histology reports from all EBUS procedures undertaken in 2016, 2017 and 2018, recording the number of patients each year and the malignancy status of each patient. In total, 270 EBUS histology reports were evaluated from 185 individual patients, with each patient having between one and four separate samples reported on. The notes of all patients with no malignancy detected on EBUS were obtained for further investigation, to determine if a subsequent diagnosis of malignancy was made.
In 2016, 13 patients underwent EBUS, of which 5 (38%) were found to have malignancy. Of those not found to have malignant features on EBUS, 75% were later diagnosed with malignancy. In 2017 there were 77 patients, with 30 (39%) showing malignancy on EBUS and 36% of the EBUS-negative patients later being diagnosed with malignancy. In 2018, there were 98 patients, with 25 (26%) showing malignancy and 53% of patients with negative EBUS later diagnosed with malignancy.
Our data demonstrates an annual increase in the number of EBUS procedures performed at the hospital from 2016 to 2018, as more Dept members became trained in the procedure. The rate of malignancy detection decreased during this time, while the false negative rate initially reduced from 2016 to 2017, but then increased to an intermediate level in 2018.

P11

Multi-Site Audit of EGFR T790M Confirmation by Liquid/Tissue Biopsies and Osimertinib Use in Patients with EGFR Mutant Non-Small Cell Lung Cancer

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Purpose of the study: NICE recommends the use of Osimertinib as second line therapy only in patients with advanced lung adenocarcinoma with confirmed EGFR T790M mutations, which mediates resistance to first and second generation EGFR inhibitors in about 50% of resistance. Liquid biopsies assessing circulating tumour-derived DNA can detect this alteration, but their sensitivity is limited by tumour burden, particularly in patients with small volume oligoprogressive disease. Previously, we presented audit results showing good adherence at West Suffolk Hospital to NICE standards for Osimertinib use. Here, we present a re-audit a year later with extension to Addenbrooke's Hospital, encompassing a larger number of patients.
Methods: We retrospectively analysed records of patients known to have received Osimertinib outside of compassionate access schemes. 20 patient records were identified and analysed (the previous audit identifying only five patients).
Summary of results: Despite a larger cohort, full adherence to NICE guidance was noted. All patients had EGFR T790M genomic confirmation: eight confirmed using liquid biopsy and 12 using tissue biopsy. Osimertinib was well tolerated and clinically effective (17/20 patients experienced clinical benefit, defined objectively as stable disease or partial response on imaging).
Conclusions: Overall, we present strong adherence to NICE standards of care and a positive experience of Osimertinib use.

P12

Genomic Abnormalities of TP53 Define a High-Risk Subgroup of Paediatric B-Cell Non-Hodgkin Lymphoma

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Purpose: Despite high cure rates for children with B-cell non-Hodgkin lymphoma (B-NHL), outcome after disease progression remains dismal. TP53 mutations are effective risk classifiers in many cancers but although common, their prognostic relevance in paediatric B-NHL remains unknown. We evaluated the clinical and biological significance of TP53 abnormalities in the largest cohort of B-NHL in children.
Methods: Diagnostic samples from 95 UK B-NHL patients were assessed for TP53 mutations and 17p alterations by genomic sequencing and copy number arrays. Clinical outcome data were collected and factors impacting survival analysed using log-rank analysis and multivariate modelling.
Results: TP53 abnormalities (mutations, deletion and/or copy number neutral loss of heterozygosity) were present in 55% of cases. TP53 abnormalities at presentation were associated with a significantly inferior time to progression (HR 14 (95% CI 1.8-100), p=0.012), which was even more pronounced in cases presenting with biallelic abnormalities (biallelic HR 16 (95% CI 2.1-130), p=0.0081). TP53 abnormalities remained an independent prognostic marker in multivariate analysis, incorporating known high-risk clinical factors (BM and CNS involvement) (time to progression HR 11.7, 95% CI 1.5-91.6, p=0.019). TP53 abnormalities were associated with complex chromosomal alterations, amongst which was a novel complex pattern on 13 (termed 13qplex), frequently involving MIR17HG which has an established role in MYC driven lymphoma. Finally, we demonstrate the maintenance or acquisition of biallelic TP53 abnormalities at relapse in all six paired diagnosis/relapse Burkitt lymphoma cases studied.
Conclusion: This study demonstrated the prognostic significance of TP53 abnormalities in paediatric B-NHL, identifying TP53 abnormality as biomarker of high-risk. Conversely, we show that patients with no TP53 abnormalities have an extremely low risk of relapse and may be candidates for treatment de-escalation.

Ⓟ = Presenter ✱ = Supported by a Pathological Society Grant

P13 *

Identifying Potential Therapeutic Targets of Aneuploidy in Classical Hodgkin Lymphoma

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Purpose of study: There is currently an unmet need for alternative treatment strategies in Classical Hodgkin lymphoma (cHL). Aneuploidy and chromosomal instability ubiquitously affects cHL and represent unexplored potential therapeutic targets. The aim of this study was to explore the effect of aneuploidy on B-cells and assess whether aneuploidy is a viable therapeutic target in primary cHL.
Methods: We used a novel experimental model in which aneuploidy is induced in cultured B-cells by treatment with CENPE and MPS1 inhibitors. Treated and untreated cultures underwent single cell RNA sequencing using the Chromium10X platform for single cell gene expression profiling (GEP). Aneuploid cells were inferred bioinformatically using our novel aneuploidy caller. We studied the overlap between genes differentially expressed in aneuploid cells (compared to euploid cells) with genes differentially expressed in primary HRS cells (compared with normal CD30+ extrafollicular B-cells).
Summary of results: In B cells induced into aneuploidy, 2051 genes were found to be upregulated compared to euploid cells. A gene ontology analysis revealed enrichment among up-regulated genes of functions that included antigen presentation (p=2.58E-09), interferon-γ signalling (p=6.91E-08) and protein stress (p=6.87E-06). 1153 genes differentially expressed in aneuploid B-cells were also differentially expressed in HRS cells accounting for 16.2% of the transcriptional profile of HRS cells.
Conclusions: Aneuploidy accounts for a significant proportion of the transcriptional programme in cHL. The findings highlight potential novel therapeutic targets in cHL including proteotoxic stress pathways via small molecule compounds and immunotherapeutic approaches.

* ***This research was supported by the Jean Shanks Foundation and the Pathological Society of Great Britain & Ireland.***

P14

Early Detection of T-Cell Lymphoma with T Follicular Helper Phenotype by RHOA Mutation Analysis

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Angioimmunoblastic T-cell lymphoma (AITL) and peripheral T-cell lymphoma with T follicular helper phenotype (PTCL-TFH) are a group of complex clinicopathological entities that are originated from TFH cells and share a similar mutation profile. Their diagnosis is often a challenge, due to lack of specific histological and immnunophenotypic features, paucity of neoplastic T-cells and prominent polymorphous infiltrate. We investigated whether the lymphoma associated RHOA mutation, occurring in 60% of cases, is present in the early "reactive" lesions and mutation analysis can help lymphoma early diagnosis. RHOA G17V [G50T] mutation was detected by quantitative PCR with a LNA probe specific to the mutation, and a further PNA probe to suppress the amplification of the wild type allele. The qPCR assay was highly sensitive and specific, detecting G17V at an allele frequency of 0.03%, but not other changes in G17, nor in 24 reactive lymph nodes. Among the 32 cases of AITL and PTCL investigated, RHOA G17V was detected in 17, of which 13 had multiple biopsies including preceding biopsies in 10 and follow up biopsies in 3. RHOA G17V was present in each of these preceding or follow up biopsies including 12 specimens that showed no evidence of lymphoma by histological, immunophenotypic and clonality analysis. The mutation was seen in biopsies 0.3-29 months (mean=10 months) prior to lymphoma diagnosis. Our results show that RHOA G17V mutation analysis is valuable in early detection of AITL and PTCL-TFH.

P15

Fine Needle Aspiration Cytology of a Rapidly Enlarging Neck Lymph Node

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A 46 year old man presented to his GP with a five week history of increasing left neck lymphadenopathy and night sweats. There was no history of weight loss or fever. He was referred to the head and neck team and an urgent fine needle aspirate (FNA) was taken from an enlarged left neck lymph node. FNA of the left neck mass demonstrated a cellular specimen of singly dispersed medium to large cells. Some of the cells were multinucleated with prominent nucleoli. The background was composed of abundant small lymphocytes, neutrophils and eosinophils. No pigment was present.
At the time of FNA a tissue biopsy was also undertaken. The malignant cells were positive for CD30 and ALK. They were negative for CD45, CD3, CD5, CD15, PAX5 and AE1/AE3. This is an ALK positive anaplastic large cell lymphoma. These are rare lymphomas that are more commonly seen in men and are known to have a better prognosis than the ALK negative subset. Patients' often report B symptoms (fever, night sweats and weight loss) and extranodal presentations have been reported. They usually have a t (2;5) translocation.
This case highlights the limited role of FNA cytology in the management of lymphomas, and specifically the diagnosis of less common forms. Rapid diagnosis with appropriate triaging of the patient is possible with cytology but in many instances further classification is not always possible. A comprehensive report outlining the differential diagnosis and degree of certainty of diagnosis is necessary and should be provided for the clinician to act upon.

P16

TRAF3 Degradation in Mesenchymal Cells Promotes Accumulation of RANKL- and TGF-beta-Expressing Immune Cells in Bone During Aging and Causes Osteoporosis

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TNF receptor-associated factor 3 (TRAF3; a negative regulator of NF-κB signaling) is degraded during aging in murine osteoblast and osteoclast precursors by TGFβ and RANKL, respectively, resulting in osteoporosis. TGFβ1+ granulocytic (TGCs; CD11b+Ly6C+6G^{hi}) and RANKL+ plasmacytoid B (RPBCs; CD19+B220^{hi}IgM+CD138+) cells are major sources of TGFβ and RANKL in bone marrow (BM) where they accumulate in WT mice during aging and in 9-m-old TRAF3$^{f/f}$/Prx-1^{Cre} (TRAF3-cKO) mice with TRAF3 deleted in mesenchymal stem cells (MSCs).
Purpose: To examine how these immune cells accumulate in BM during aging.
Methods and Results: CD45- MSCs were sorted from BM from aged WT (24-m) and 8-m-old TRAF3-cKO and WT mice. *Ccl5* and *Cxcl12* mRNA and protein levels were significantly higher in microarray screens of BM from 24-m WT and 8-m TRAF3-cKO than from 8-m WT mice. NF-κB RelA and RelB binding to the *Ccl5* and *Cxcl12* promoters was higher in TRAF3-cKO than in WT MSCs. *Ccl5* and *Cxcl12* mRNA levels were higher in vertebral samples from 20 elderly humans than from 20 children ($p<0.01$). 85±3% of TGCs and 97±1% of RPBCs expressed CCR5 (CCL5 receptor) and CXCR4 (CXCL12 receptor), respectively. Inhibitor of apoptosis proteins (IAPs; E3 ligases), mediated TGFβ1-induced TRAF3 degradation, and IAP protein levels were higher in bone of aged (20-m) than young WT (3-m) mice. The IAP inhibitor, SM164, prevented TGFβ1-induced TRAF3 degradation in vitro. Injections of SM164 or the FDA-approved drugs, Plerixafor (P), a CXCR4 antagonist and HSC mobilizer, or Maraviroc (M), a CCR5 antagonist and anti-HIV drug, for 4 wk inhibited accumulation of TGCs and/or RPBCs in BM of 22-m-old mice and increased vertebral bone mass (BV/TV: 14±2% with SM164, 15±3% with P, 15±3% with M vs. 11±2% in Ctrl; $p<0.05$).
Conclusion: TGFβ1-induced TRAF3 degradation in MSCs, mediated by IAPs, promotes CCL5 and CXCL12 expression and accumulation of RPBCs and TGCs in BM to cause bone loss with age. FDA-approved P and M could prevent osteoporosis.

P17

Complex Rearrangements Drive Tumorigenesis in Some Mesenchymal Tumours

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Purpose of the study: The canonical *EWRS1*-ETS family fusions in Ewing's sarcoma were recently found to result from complex rearrangements, spanning multiple chromosomes and resembling the punctuated rearrangement event, chromoplexy. We explored these phenomena in other mesenchymal tumours. MethodsWe subjected an osteoblastoma and infantile fibrosarcoma (IFS) to whole genome sequencing and identified respective *PPP1R10-FOSB* and *NTRK3-ETV6* fusions, both validated by RNA sequencing. Copy number and rearrangements calls were manually reviewed and reassembled to construct a map of each complex rearrangement.
Summary of results: The osteoblastoma genome possessed an elaborate cycle of rearrangements that spanned five chromosomes. Each chromosome contained a pair of breakpoints within close proximity and in or nearby five genes, namely *FOSB, PPP1R10, TSC22D1, NPIBP4, and TTC7B*. There was no associated genomic loss, demonstrating conservative repair with erroneous reassortment of five simultaneous chromosomal breaks; the definition of chromoplexy. The IFS genome contained breakpoints pairs on three chromosomes. Breakpoint pairs on chromosomes 15 and 12 were separated by genomic gains of approximately 1MB. Each contained a breakpoint within a gene, *NTRK3* and *ETV6* respectively, generating the fusion. A further pair of breakpoints were within close proximity on chromosome 13. The larger inter-breakpoint distances prevented the unambiguous reconstruction of derivative chromosomes. This complex event could either represent insertion of templated sequences from chromosome 12 and 15 or a chromoplexy event with staggered DNA breaks with large duplications during repair.
Conclusions: These findings are part of an emerging picture that complex rearrangements underpin many fusions in mesenchymal tumours. These complex events generate driver mutations, define a critical step in tumorigenesis, and may confound diagnostic techniques. Their frequency and mechanism warrants further study.

P18

The Genomic Patterns of Metastatic and Malignant Progression of Giant Cell Tumours of Bone

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Purpose of the study: Giant cell tumours of bone (GCT) are defined by G34 mutations of the H3.3 histone genes. We set out to explore the genomic relationships between conventional, malignant and benign metastasising GCT.
Methods: We conducted whole genome sequencing on 7 GCTs and 7 malignant bone tumours with an H3.3 G34 mutation. We also sequenced the primary and 2 metastases of a benign metastatic GCT. We conducted methylation array analysis on 42 GCTs, 15 malignant H3.3 mutated tumours, 42 osteosarcoma (as malignant non-H3.3 mutated tumours) and 19 chondroblastoma (benign tumour with a H3.3 K36M mutation).
Summary of Results: Conventional GCTs possessed no further driver mutations beyond an H3.3 mutation. All malignant H3.3 mutated tumours developed at least one additional driver mutation: 5/7 tumours acquired replicative immortality by either *TERT* promoter mutation or enhancer highjacking, or Alternative Lengthening of Telomeres (ALT), 2/7 tumours acquired an epigenetic modulator mutation in a histone Lysine Specific Demethylase (*KDM4B/6A*). 3/7 malignant tumours were genome duplicated, with substantial aneuploidy and chromothripsis. Timing analysis demonstrated that H3.3 mutation preceded genome duplication which itself occurred several years prior to diagnosis. A multi-sample benign metastatic case had no additional drivers or aneuploidy, but its metastases were polyclonally seeded. Unsupervised clustering of methylation profiles segregated chondroblastoma, osteosarcoma and benign and malignant H3.3 G34-mutated bone tumours, though the latter two classes were closely related. *CCND1* promoter methylation status segregated benign and malignant G34-mutated tumours.
Conclusions: H3.3 mutations precede copy number aberration in malignant tumours, which suggests they represent malignant GCTs. Conventional "benign" metastatic and non-metastatic GCTs are indistinguishable by genomic or methylation profiling, supporting the prevailing passive metastasis hypothesis.

P19 ✱

Single-Cell Analysis of Cancer-Associated Fibroblast Heterogeneity in Non-Small Cell Lung Cancer: Relating Molecular Phenotype to Function

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CAF remain a poorly-characterised population, despite their abundance in most solid cancers. No single molecular marker identifies all CAF, and there has been a scarcity of evidence regarding the existence of distinct subtypes and whether such subgroups have different functions. This work aims to characterise the heterogeneity the cancer-associated fibroblast (CAF) population in non-small cell lung cancer. Fresh human lung tissue was dissociated for sixty minutes to extract the maximum possible proportion of fibroblasts. Single-cell RNA sequencing (scRNA-seq) was performed using a droplet-barcoded platform (Drop-seq). Quality control was performed on the raw sequencing data and resulting gene expression matrix. Bioinformatic analysis was performed using multiple packages in R. Spatial relationships between cell types were assessed using a multi-immunohistochemical (IHC) staining technique. We developed a workflow for efficient processing of raw Drop-seq data including quality control, normalisation and visualisation. We applied this method to samples from twelve non-small cell lung cancer (NSCLC) patients, integrating the resulting data with that from an existing NSCLC scRNA-seq dataset. Our analysis identified 9 stromal cell subtypes: 3 predominantly derived from normal tissue and 6 largely from tumour samples. Of the normal subtypes, one showed gene expression consistent with the previously-described "inflammatory" fibroblast phenotype. Trajectory analysis identified a branched differentiation process from normal to CAF phenotypes, suggesting that these cells share a common initial activation before differentiation to distinct subtypes. These populations also show differential gene set enrichment, indicative of functional differences. In keeping with this, the phenotypes show distinct prognostic impact across NSCLC subtypes.
✱ ***This work is supported by a Pathological Society of Great Britain & Ireland Trainees' Small Grant.***

P20

In Silico Analysis of Single Nucleotide Polymorphisms (SNPs) in Human FOXC2 Gene

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Lymphoedema is an abnormal accumulation of interstitial fluid, due to inefficient uptake and reduced flow, leading to swelling and disability, mostly in the extremities. Hereditary lymphoedema usually occurs as an autosomal dominant trait with allelic heterogeneity. This study aimed to identify the mutations which are most likely to affect the function of Forkhead box C2 (FOXC2), leading to the development of lymphoedema distichiasis syndrome (LDS). We identified single nucleotide polymorphisms (SNPs) in the FOXC2 gene using dbSNP, analyzed their effect on the resulting protein using VEP and Biomart, modelled the resulting protein using Project HOPE, identified gene — gene interactions using GeneMANIA and predicted miRNAs affected and the resulting effects of SNPs in the 5′ and 3′ regions using PolymiRTS. We found 473 SNPs associated with FOXC2 - 429 were nsSNPs and 44 SNPs were in the 5′ and 3′ UTRs. In total, 2 SNPs - rs121909106 and rs121909107 - have deleterious effects on the resulting protein, and a 3D model confirmed those effects. The gene—gene interaction network showed the involvement of FOXC2 protein in the development of the lymphatic system. hsa-miR-6886-5p, hsa-miRS-6886-5p, hsa-miR-6720-3p, which were affected by the SNPs rs201118690, rs6413505, rs201914560, respectively, were the most important miRNAs affected, due to their high conservation score. Rs121909106 and rs121909107 were predicted to have the most harmful effects, while hsa-miR-6886-5p, hsa-miR-6886-5p and hsa-miR-6720-3p were predicted to be the most important miRNAs affected. Computational biology tools have advantages and disadvantages, and the results they provide are predictions that require confirmation using methods such as functional studies.

Ⓟ = Presenter ✱ = Supported by a Pathological Society Grant

P21

The N6 Consortium: The Northern Pathology Network

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The Northern Pathology Imaging Collaborative (NPIC), is an Innovate UK-funded, industrial research collaboration. The N6 Consortium, a work-package within NPIC, is a network of academic pathologists, based in the North of England. Underpinned by Roche Tissue Diagnostics, the N6 will develop and evaluate Roche AI algorithms, to enhance precision medicine.

The main study will demonstrate the feasibility of the prospective and retrospective recruitment of 960 colorectal cancer patients, all of whom have received anti-EGFR monoclonal antibody therapy (either cetuximab or panitumumab). Formalin-fixed, paraffin-embedded (FFPE) tumour samples will be stained for three biomarkers (amphiregulin [AREG], epiregulin [EREG] and EGFR). Expression levels will be determined using Roche AI algorithms, with subsequent evaluation of its use in predicting response to anti-EGFR therapy. A second pilot study will examine particular immunohistochemical markers in lung cancer, including PDL1 and a third pilot will investigate protein biomarkers in breast cancer, including Her2. To date, training sessions on the use of the Benchmark ULTRA instruments have been carried out, and a VENTANA DP 200 slide scanner has been installed at each of the N6 sites. A pre-study, cross-site Quality Assurance (QA) programme will be undertaken, to ensure that both the staining and scanning at each N6 site, is comparable.

The development of this Consortium, will aid the growth of a Northern network of academic Pathology sites, and allow improved integration with the Oncology community, thus providing a much needed framework for collaborative research in the future.

P22 *

Overexpression of MicroRNA 21 Leads to Increased Glial Scarring in Spinal Cord Injury

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Spinal Cord Injury (SCI) results in major functional neurological impairments with little to no recovery. The cellular events that are triggered from the injury such as apoptosis, the inflammatory process and the formation of the glial scar lead to the majority of the functional deficits. These cellular processes are controlled by gene expression within cells, which in turn can be regulated by epigenetic microRNA molecules (miRNAs). MicroRNA-21 (miR-21) is highly expressed after SCI and may have an important role in the subsequent pathophysiological or repair mechanisms. Mice with global overexpression of miR-21 (OE) (n=6) or global knockdown of miR-21 (KO) (n=5) underwent SCI and histology of the spinal cord lesion were compared to wild-type (WT) (n=6) mice. Immunohistochemistry was performed on the injured spinal cords to study the astroglial response with the biomarker glial fibrillary acidic protein (GFAP) for reactive astrocytes; microglial infiltration with the biomarker ionized calcium binding adaptor molecule 1 (Iba1) for activated microglia; and glutamatergic axon survival with the biomarker vesicular glutamate transporter 1 (VGLUT1). MiR-21 OE mice were found to have significantly increased GFAP staining at the lesion site, with 66% (p=0.0198) and 116% increase (p=0.0017), as compared to miR-21 KO and WT mice respectively. MiR-21 OE mice showed a trend towards increased Iba1 staining at the lesion compared to miR-21 KO and WT mice, although this did not reach significance. MiR-21 KO resulted in a non-significant reduction of almost 50% in VGLUT1 staining. The increased glial scar formation that is modulated by miR-21 may therefore have an important role to play in axon survival and regeneration by providing a permissive environment for axonal growth. Further research will uncover insight into the therapeutic potential of miR21 after a SCI to promote recovery.

* ***This research was supported by a Pathological Society of Great Britain & Ireland Grant.***

P23 *

Genotyping TBXT in Patients with Benign Notochordal Cell Tumours: My Experience as a Summer Student

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Purpose of the study: This past summer, I won a place in the new Pathology Summer Studentship Scheme. The aim of this 6-week placement was to learn more about medical research and pathology. My project was to determine if there was a germline genetic correlation between benign notochordal cell tumours (BNCTs) and the SNP rs2305089 in TBXT (aka brachyury). I also wished to learn how to answer questions about the cause of disease using different cell and molecular techniques. BNCT and chordoma are exceptionally rare tumours which share similar anatomical and histological features with the notochord. The diagnostic hallmark is the expression of the gene TBXT. Whereas approximately 45% of the European population have a SNP rs2305089 in this gene 95% of patients with chordoma harbour this variant.
Method: Following DNA extraction, quantification and quality control from blood and buccal samples from individuals with BNCT I performed Taqman qPCR assays. I then interrogated the genotype at rs2305089 from 21 patients with BNCT.
Results: I found that the frequency of the mutant allele was 69%.
Conclusion: The cohort studied was too small from which to draw any conclusions. The team is continuing to collect samples from this rare patient group. The Studentship also provided the opportunity to put into practice techniques I had only learnt in theory including making tissue microarrays, and gene editing using CRISPR of inducible pluripotential stem cells to see how the SNP rs2305089 modifies the gene expression TBXT: these experiments are on-going. I attended the weekly research lab meetings and the Path Soc meeting in Harrogate. I met patients with chordoma. This Studentship although short exposed me to research and the speciality of pathology, two areas vital in medicine, yet lacking in the current medical curriculum.

* ***This work was performed as a Summer Student in UCL Research Department of Pathology and was funded by Prof AM Flanagan and the Pathological Society of Great Britain & Ireland.***

P24 *

Development of an Optimal Set of Post-Processing Filters for Hybrid Capture Next Generation Sequencing (NGS) Experiments on FFPE Derived Tumour DNA: Summer Student Project

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Background: NGS studies are now routinely used for the detection of novel and clinically actionable DNA variants in clinical practice to enable personalised medicine. Formalin-fixed paraffin-embedded (FFPE) tissues are still the mainstay for tissue diagnosis but the fixation process is known to degrade DNA and therefore compromise further analysis.There is still a lack of adequate informatic tools to filter out the DNA artefacts from FFPE tissues.
Aim: To develop a set of post-processing filters in R for the identification of novel and hotspot mutations in translational cancer research experiments.
Methods: 150bp paired end sequencing was performed on 96 sarcomas with matching normal DNA.The hybrid capture design comprised the exons of 250 cancer genes, 96 genotyping SNPs and a SNP backbone for copy number estimation. Data comprising BAM and VCF files. Bioinformatic packages used included BWA-mem, Mutect2, vcf2maf and ggplot2. Bespoke R scripts and bash scripts were created. Manual curation was performed using the intergrative genome browser. Based on these results of sequencing quality and depth a set of post processing filters was developed.
Results: The normal tissues were sequenced to a median depth of 212X and the tumours 343X. The mean PCR duplicate rate was 30% and the on target capture rate was on average 60% in the tumours. The total number of variants across the dataset was 333294 but decreased dramatically to 6234 after applying the post-processing filters. Manual curation of variants revealed that a significant percentage of mutations were being called in chimeric reads requiring re-iteration of the filters and a rerun of the QC process.
Conclusions: The impact of formalin on DNA quality and mutation calling is significant. However, the identification of true positive mutations can be achieved by the development of appropriate post-processing filters.Future work includes validation of novel variants and re-iteration of the filters for future studies.

* ***This work was performed as a Summer Student in UCL Research Department of Pathology and was funded by Prof AM Flanagan and the Pathological Society of Great Britain & Ireland.***

P25 *

Characterisation of Lentiviral Accessory Protein Vpx as an NF-κB Antagonist

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Purpose of study: To investigate a new role of lentiviral accessory protein Vpx in antagonism of the NF-κB and IRF3 inflammatory pathways in DNA sensing.
Methods: Cell-line luciferase reporter assays were carried out in 293T cells reconstituted for DNA sensing molecules and transfected with Vpx or Vpx mutants. Immunoprecipitation experiments in 293T cells were carried out with over-expressed Vpx and NF-κB signalling pathway members. Cell-line reporter assays were also carried out in DCAF1 knockdown cells, achieved by transient knock-down with siRNAs.
Summary of results: Cell-line reporter assays showed Vpx directly antagonises NF-κB, but not IRF3, activation downstream of DNA-sensing. NF-κB antagonism was preserved in Vpx binding mutants described in the literature. Immunoprecipitation experiments showed that Vpx interacts with several NF-κB signalling molecules and likely targets key NF-κB subunit p65 for degradation. Transient knockdown experiments indicated Vpx antagonism of NF-κB is independent of host co-factor DCAF1.
Conclusions: Our experiments suggest a novel phenotype of Vpx to antagonise NFκB signalling, distinct from previously described roles of the protein. Notably, Vpx antagonism of NF-κB is independent of host co-factor DCAF1, representing an entirely novel mechanism of Vpx action. We believe this phenotype may have implications for evasion of DNA-sensing and host immune responses *in vivo* which warrant further investigation in the context of infection.

* ***This study was supported by the Pathological Society Undergraduate Bursary.***

P26

Molecular Characterisation of Paired Head and Neck and Lung Squamous Cell Carcinoma Aids in Distinguishing Pulmonary Metastases from a New Primary Lung Cancer

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Purpose of the study: Lung squamous cell carcinomas (LSCC) and head and neck squamous cell carcinomas (HNSCC) may present as synchronous or metachronous tumours. They share common pathogenesis, microscopic and molecular features, making differential diagnosis on metastatic sites highly challenging. Currently, the differentiation between lung metastasis from HNSCC and a second primary LSCC is made on clinical and histologic criteria which are not very accurate. This distinction is crucial as the choice of therapy is usually different in these situations. Molecular characterization could be useful in this setting. The aim of the study was to identify molecular signatures to aid in distinguishing between these clinical scenarios.
Methods: We identified 16 cases of HNSCC that developed subsequent LSCC. Four paired samples from the head and neck and lung tumours had sufficient material and were subjected to mutational analysis by targeted next-generation sequencing (Ion torrent, Thermofisher) using a '53' gene cancer hot-spot panel.
Results: Mutational analysis favoured separate primaries in one patient and metastatic disease in two patients. The two patients with metastatic disease had identical mutations in the TP53 and CDKN2A genes in primary and metastatic site. A conclusive distinction was not achieved in one of the four patients.
Conclusions: Our cases demonstrate an important adjunct role of molecular characterization in separating metastatic disease from development of a second primary cancer, thus aiding in clinical management of these patients. In addition, as smoking related-HNSCC and LSCC share common molecular features, whole-exome/genome sequencing studies on a larger cohort may be required to identify specific molecular signatures to further distinguish between primary and metastatic disease.

P27

Bioinformatic Analysis of Histopathological Variables for Prognostic Stratification of Melanoma

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Purpose of study: Considering the increasing prevalence of melanoma globally, prediction of survival using evidence-based melanoma staging systems is key to improving survival rates. Currently, there are a number of histopathological variables with conflicting research surrounding their prognostic relevance. Therefore, bioinformatic analysis of these variables using large melanoma patient databases is vital. It was hypothesised that hierarchical clustering, univariate and multivariate analysis of pathological variables would help identify key melanoma prognostic markers.
Methods: A retrospective cohort study was conducted allowing the construction of a melanoma database. Patients with statistically sufficient pathological data (n=557) were used to carry out hierarchical clustering, alongside Kaplan-Meier and log rank methods. This allowed determination of poor, moderate and good prognostic clusters. Variables within each cluster were analysed using Chi-Squared for trend to comprehend the variables defining each cluster and their correlation with prognosis. Further univariate and multivariate analysis was conducted to compare significant prognostic variables for this cohort and a potential melanoma prognostic index was developed.
Summary of results: The major finding from this study was that mitotic rate, a variable not currently used in the AJCC8 staging system, showed independent prognostic significance for this study cohort. Furthermore, ulceration and microsatellites were not significant in multivariate analysis, which challenges their current use in staging systems.
Conclusions: It is clear from this study that the re-evaluation of these variables for inclusion in future melanoma staging systems is paramount. Further bioinformatic analysis in larger patient cohorts is vital for validation. Nonetheless, this research holds great potential to shape future melanoma staging systems and improve prediction of clinical outcomes.

P28

The Use of Bioinformatics in Identifying Prognostic Groups in Malignant Melanoma

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Purpose of the study: To investigate if the combination of all prognostic factors can be used as a predictor of distant metastasis-free (DMF) and overall survival (OS) in the melanoma cohort. Besides that, this study is to determine if the response to immunotherapy by clustering immunotherapy-receiving patients according to their relapse-free (RF) survival can be predicted.
Methods: Patients who were diagnosed with primary melanoma were reviewed retrospectively and divided into four clusters according to either distant metastasis-free or relapse-free after receiving immunotherapy were encountered. Then, univariate analyses were used to identify which prognostic factors were predictors of distant metastasis-free, relapse-free and overall survival.
Summary of results: There were two significant prognostic factors that acts as predictors of patient's distant metastasis-free and overall survival; (1) Breslow thickness ($p<0.001$) and (2) age ($p<0.001$). In both DMF and OS survival curves, cluster 2 and 3 shown distinct separation between them with five significant prognostic factors; age ($p<0.001$), site of primary tumour ($p<0.001$), Breslow thickness ($p<0.001$), ulceration ($p<0.001$) and mitosis ($p=0.015$). Cluster 2 and 4 also have shown distinct separation between their survival curves with two significant prognostic factors; age ($p<0.001$) and ulceration ($p=0.026$). There is only one independent prognostic factor that has significance towards predicting relapse-free which is microsatellites where $p=0.021$.
Conclusion: Age at primary diagnosis has shown to be the common significant predictor for distant-metastasis free and overall survival while microsatellite is the only significant predictor of relapse-free survival for immunotherapy-receiving patients in the melanoma cohort.

P29

OX40 Expression in Melanoma and its Relation to Other Immune Checkpoints

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Purpose of study: OX40 and associated immunogenic markers are expressed on tumour immune cells. These cells can be harnessed by immunotherapeutic drugs to target melanoma cells, hence killing the tumour. This study aims to discover the prognostic significance of OX40 and its association with other immune markers. The relation between OX40, clinicopathological factors and the patient survival were tested individually and in combination with other markers.
Methods: Using IHC for OX40, CD4, CD8 and other markers were scored dependent on the number of positive cells present in a sample, from the tissue microarray (TMA). Primary and metastatic malignant melanoma cases were used from patients from our hospital. The prognostic effect that OX40, CD4 and CD8 together with previously assessed immune markers was analysed using survival curves, Spearman's correlation, chi-squared test and fisher's test.
Summary of results: OX40 on its own, did not have a significant effect on patient survival, whereas high expression of markers CD4 and CD8 were seen to increase survival (p-values were 0.013 and 0.012 respectively). When patients were clustered in groups dependent on their immune marker expression, we see patients who had higher levels of immune expression, had a greater outcome to melanoma.
Conclusions: If OX40 is to be used as an immunotherapy, it would be advisable to be targeted in combination with other markers such as CD4 and CD8. These markers are both correlated with OX40, so a bispecific antibody would be likely to induce a potent immune response against melanoma.

P30

Detection of BRAF V600E Mutation in Melanoma: Comparison Between Immunohistochemistry and Three Gene Mutation Detection Methods

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BRAF^V600E is a 'hotspot' somatic mutation which is found in 50% to 70% of melanoma patients. Detection of BRAF^V600E mutation is extremely important and necessary for early screening, diagnosis and prognosis of malignant melanoma. Genetic detection techniques have been performed as reference tools to determine mutation. More recently, immunohistochemistry (IHC) becomes a popular method to detect this point mutation by using anti- BRAF^V600E antibody binds with corresponding protein. In order to evaluate whether IHC can replace genetic methods to detect BRAF^V600E mutation, this study have selected 100 metastatic melanoma patients to detect BRAF^V600E mutational status by two PCR-based methods combine with previous available pyrosequencing data to compare with IHC. 364 patients were available to assess the relation of DNA-based PCR methods and IHC. Among these patients, 42.6% of patients were found to have BRAF^V600E mutations with PCR and 37% with IHC test. In this study, the IHC displayed a specificity of 95.7% and sensitivity of 87.2%. Negative predictive value (NPV) is 90% and positive predictive value (PVV) of 93.8% (335/364). And the concordance rate is 92.0%. Moreover, some clinicopathological features including age, site and melanoma subtype exhibited significantly association (P<0.05) with BRAF^V600E mutational status detected by IHC and genetic methods. Consequently, this study suggested that IHC is cost-effective, suitable and reliable first-line methods to screen for BRAF^V600E mutant melanoma. Thereafter, genetic methods can be used when ambiguous samples are obtained.

P31

Audit of Sentinel Lymph Node Biopsy in Melanoma Cases from a Single Institution

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Purpose of the study: Patients with cutaneous melanoma between 0.8mm and 1mm thick are recommended to undergo sentinel lymph node biopsy (SLNB) as part of their staging. The purpose of our study was to compare our institutions rate of positive SLNB and reported positive rates.
Methods: Patients with cutaneous melanoma who underwent SLNB at our institution between January 2017 and July 2019 were identified using our laboratory system.
Summary of results: 111 patients were identified. 21 cases were excluded. This resulted in a total of 90 eligible cases, 58% female and 50% <60 years of age.
52% of cases were superficial spreading subtype, 40% nodular, 4% acral lentiginous and 2% lentigo maligna melanoma. 88% had dermal mitoses (> or equal to 1 mitosis present). 61% had a Breslow thickness of greater than 1mm. 80% had no ulceration.
61% were Clarks level 3, and 28% Clarks level 4.
39% were stage pT1 and 33% were pT2.
40% of cases involved the lower extremities, with 28% involving the upper extremities.
14% had positive SLNB. Of these patients, 54% proceeded to lymph node clearance, with 43% having additional positive lymph nodes.
1/5 cases with BT <0.8mm had positive SLNB, and 3/30 (10%) with BT between 0.8-1mm had positive SLNB, giving 11% positive SLNB in patients with thin melanoma (<1mm).
62% were Clarks level 4 or 5.
Conclusions: Our rate of positive SLNB in thin melanomas (<1mm) is 11% which is in keeping with international reported rates. Features associated with positive SLNB were age younger than 60 years, female, greater than 0.8mm thick, presence of dermal mitoses and nodular subtype.

P32

Orange Discolouration of the Skin Associated with the Use of Pazopanib for Treatment of Metastatic Renal Cell Carcinoma.

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Purpose of the study: To describe the first reported case of skin discolouration due to the use of Pazopanib, a multi-targeted tyrosine kinase inhibitor with anti-angiogenic properties used in renal cell carcinoma.
Methods: The clinical and the histopathological features of the affected skin are described. This report describes the case of a 65 year old gentleman with a background history of metastatic renal cell carcinoma treated with Pazopanib, leading to large cutaneous bilateral orange patches on both flanks, eighteen months after commencing Pazopanib. Skin biopsy showed a dermis with an infiltrate of macrophages, which contained granules within their cytoplasm. These granules were non polarizable on Haematoxylin and Eosin stained sections. There was no inflammatory reaction around these foci. Multinucleated giant cells were not a feature. Pazopanib was discontinued, soon after the discolouration was noted to resolve.
Summary: Dermatological side effects are among the most frequently encountered toxicities associated with targeted anti-cancer medications, as the pathways blocked by these medications are also vital in maintaining cutaneous function. These include skin eruptions, pruritus, photosensitivity, vasculitides, hand and foot skin reactions, xerosis, fissures, alterations in hair and nails and pigmentary changes. While hypopigmentation of the skin has been described with Pazopanib use, this is the first reported case of associated discolouration.
Conclusion: It is important that clinicians provide a history of a patient's medication use in laboratory request forms. Pathologists should also be aware of adverse cutaneous effects following the use of targeted drug therapy to allow for correct diagnosis. This will facilitate the provision of patient education, as these side effects are typically reversible once the medication has been discontinued.

P33

A Cutaneous/Subcutaneous Pleomorphic Liposarcoma which was Diagnosed Only on its Recurrence – A Rare Sarcoma in a Rare Site with an Excellent Prognosis

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Purpose of study: To describe an undifferentiated cutaneous/subcutaneous myxoid and spindle cell sarcoma which on recurrence showed lipoblasts thus allowing a diagnosis of a pleomorphic liposarcoma (PLS).
Methods: We performed a review of the histopathology database in our hospital and identified one case of cutaneous/subcutaneous pleomorphic liposarcoma (CSPLS).
Summary of results: A 58-year-old female presented with a six month history of swelling of the forearm. Following removal histopathology showed a myxoid and pleomorphic spindle cell tumour without lipoblasts. Amongst a wide panel of immunohistochemical markers only CD34 showed reactivity. Despite a wider re excision the lesion recurred 18 months later and showed features of a pleomorphic liposarcoma. The tumour was less myxoid on this occasion and showed pleomorphic spindle cells but also now demonstrated numerous lipoblasts. A diagnosis of cutaneous/subcutaneous pleomorphic liposarcoma was made.
Conclusion: A literature review for cutaneous or subcutaneous pleomorphic liposarcomas was performed. Fifteen cases were found, 3 of which occurred in the forearm. Unlike the outcome of PLS at other sites with up to a 50% metastatic rate none of the cutaneous or subcutaneous liposarcomas metastasised and none were associated with disease related death. This case of PLS occurred in a most unusual location and initially had no differentiating features of PLS. The case highlights the importance of considering a diagnosis of PLS in all pleomorphic or high grade sarcomas of the cutanaeous and subcutaneous sites. In all such cases wide sampling is advised. PLS despite being an aggressive sarcoma in other sites behaves in an indolent manner in a cutaneous or subcutaneous location if adequately excised.

P34

Immunological Cluster of Primary and Metastatic Malignant Melanoma and Their Relation to Patient Survival and Response to Immunetherapy

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Malignant Melanoma (MM) is infamous as the most aggressive form of skin cancer. Management of MM is difficult once it metastasises. Presently though many treatment options are available, these adjuvant therapies have failed to improve patient survival rates and control tumour progression in advanced melanoma. The current spotlight is on immune checkpoint inhibitors as an effective anti-tumour treatment. Despite this new development, patient survival has not shown significant improvement.
The study hypothesised that the patient survival and prognosis is driven by a defined combination of immune markers with varying expression levels. We also explored the interactions between the immune clusters and their association with patient survival and clinicopathological parameters. In the study, immunohistochemistry techniques were applied to TMA cores of 813 cases of MM to analyse the combined expression profile of panel of immune markers known to be present at melanoma micro-environment. Hierarchical clustering methodology was employed to form groups of distinct immune clusters in primary and metastatic MM. Three groups with distinct expression pattern were identified for primary cohort. In metastatic cohort, many small groups were observed, making it difficult for statistical analysis. Hence, a dendrogram for the entire study population was drawn. Kaplan-Meier survival curve showed significant difference in survival ($p<0.001$) for this cluster. For the primary cluster, no significant difference was observed in survival analysis.
We conclude that intense T-cell infiltration, presence of Langerhans cells and absence/few B cells model depicts good prognosis and better survival. Also such a panel when incorporated in diagnosis could act as a key decisive factor in prognosis and response to immunotherapy.

P35

Detection Rates of Perineural Invasion in Cutaneous Squamous Cell Carcinoma

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Perineural invasion is a site specific prognostic factor and core data item in the Royal College of Pathologists' dataset for the reporting of cutaneous squamous cell carcinoma. The presence of perineural invasion (specifically defined) upstages a cutaneous squamous cell carcinoma from pT1 and pT2 to pT3 (TNM8). Published data indicates that perineural invasion is present in 2.4% to 14% of primary cutaneous squamous cell carcinoma. We audited 330 consecutive cases (July 2017) of cutaneous squamous cell carcinoma to assess the local detection rate of perineural invasion. The local detection rate of perineural invasion was 1.8% (0.6% lower than suggested published rates). This was brought to the attention of all dermatopathology reporting staff with a suggestion to be more vigilant when looking for perineural invasion and carefully consider if further work (levels/immuno) is required. A re-audit was done in August 2018 of 330 cases and this showed no change in detection rates of perineural invasion, with a local reported rate of 1.8%. In an attempt to improve our local detection of perineural invasion, we plan to do a prospective study on all cutaneous squamous cell carcinoma involving performing S100 immunostaining to identify nerves and look for perineural invasion. This will enable us to decide if our local detection rate of perineural invasion is indeed below published rates or if the local rate of perineural invasion is indeed a true variant of the published data.

P36

Accelerated Antigen Instability Testing Reveals Quantitative Mass Spectrometry Analysis Overcomes Specimen Limitations Associated with Diagnostic PD-L1 Testing

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Introduction: Immunohistochemistry (IHC) in formalin-fixed, paraffin embedded tissue (FFPET) is widely used in clinical and research settings, but has limitations relating to epitope masking, post-translational modification and immunoreactivity loss that occurs in stored tissue by poorly characterized mechanisms. PD-L1 IHC is particularly susceptible to epitope degradation and is an ideal model for understanding signal loss in stored FFPET.
Methods: We assessed 1,124 tissue sections to understand environmental factors contributing to immunoreactivity loss in stored tissues. PD-L1 IHC using 4 clones (22C3, 28-8, E1L3N, SP142) was assessed in stored FFPET of lung and gastric carcinoma. Accelerated aging of FFPET was achieved using increased humidity, oxygen and temperature. Quantitative mass spectrometry (MS) was used alongside IHC for quantifying PD-L1. Global proteome MS analyses were used to assess proteome-wide oxidation.
Results: MS quantification of PD-L1 correlated strongly with IHC expression on unaged sections ($R2=0.745$ $P<0.001$), with MS demonstrating no loss of PD-L1 protein, even in sections with significant staining loss by IHC. 22C3 and 28-8 were most susceptible to signal loss, with E1L3N the most robust (56%, 58% and 33% reduction $p<0.05$). Increased humidity and temperature resulted in significant acceleration of immunoreactivity loss, which is largely mitigated by the use of desiccants. MS demonstrated a significant but only modest oxidation of proteins by global analyses, including PD-L1.
Discussion: Immunoreactivity loss appears to be largely driven by the presence of humidity and temperature, which may result in structural distortion of epitopes rendering them unsuitable for antibody binding following epitope retrieval. Limitations of IHC for biomarker analysis in stored tissue sections can be complemented through use of MS. In some situations, MS may be preferred for retrospective analyses of archival FFPET collections.

P37

Studying Lung Cancer Heterogeneity Research Through Multiplex Imaging: A Pathology Summer Studentship Experience

Ⓟ LB Bejan

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Introduction: I won a Pathology Summer Studentship and worked on the TRACERx Lung Project (TRAcking Cancer Evolution through therapy (Rx). The aim of the multi-centre TRACERx study is to determine how clonal tumour evolution impacts upon response to therapy and clinical outcome in lung cancer patients. The study will recruit 842 patients with resectable primary non-small cell lung cancer and multi-regional sample their tumours for whole exome sequencing. Patients who relapse are rebiopsied and from this data genetic changes are used to map the course of the disease so that it is possible to identify the lethal subclone from a primary tumour which causes metastatic disease. Multiregional samples will also undergo multiplex imaging for which accurate cell segmentation will be critical for automated analysis.
Method: I played a key role in building Tissue Microarrays and acquiring lab-based skills such as slide preparation. When imaging tumour region so, the positions and boundaries of individual cells must be determined to infer cellular neighbouring, this is known as 'cell segmentation'. Determining the accuracy of automated cell segmentation requires comparison to a manually labelled ground truth image. I labelled a number of pilot lung cancer images using a computer interface devised by the team and labelled the boundaries of several distinct cell types (tumour, lymphocyte, stromal cells) and artefacts in images so that the automated segmentation of individual cell types can be tested independently. I designed the appropriate preprocessing strategy to apply to images in advance of manual labelling to make the task as easy as possible without compromising the information about the cell boundaries.
Result: The cell segmentation process developed will be used to label more sets of images to reduce any potential bias from relying on a small training set.
Conclusion: Being directly involved in a landmark study of evolution in cancer was truly an unforgettable experience.
This work was performed as a Summer Student in UCL Research Department of Pathology and was funded by Definiens, The Tissue Phenomics Company.

P38

Cancer Predisposition Syndromes: An Important Consideration in Paediatric Oncopathology

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Purpose of the study: This report highlights the significance of cancer predisposition syndromes in paediatric oncology and the importance of the pathologist's role in their recognition and diagnosis.
Case: Our patient is a 16 year old boy, who in 2011 at age eight years was noted to have café au lait patches. His brother, at that time aged nine years, presented with similar findings. Clinically, they were diagnosed as having neurofibromatosis type 1 (NF1) and surveillance was commenced as per guidelines. In 2013, his older brother developed and later died from glioblastoma multiforme. In 2018 he presented with a neck mass, on biopsy diagnosed as diffuse large B cell lymphoma. Staging CT revealed an intracranial lesion, not biopsied and multiple intra-abdominal masses, assumed to represent further lymphoma deposits. Following lymphoma chemotherapy, which led to complete remission, he was investigated for a recent history of loose stools. On colonoscopy, three rectosigmoid polyps were identified. Two were biopsied to show adenomatous features. This observation, of few colorectal polyps in a young patient with his background, prompted consideration of a cancer predisposition, in this case of constitutional mismatch repair-deficiency syndrome (CMMR-D). Immunohistochemical staining for mismatch repair proteins was performed showing complete loss of MSH6 antibody reactivity. Confirmatory genetic testing identified a homozygous pathogenic variant (c.2731C>T) in *MSH6*, consistent with a diagnosis of CMMR-D.
Conclusions: Patients with CMMR-D may present with café au lait patches, prompting clinical diagnosis of NF1. NF1 is also far more commonly encountered (with an incidence of one in 2600 to 3000), than CMMR-D, which has an incidence of only one in one million. As pathologists, we are well placed to aid in the timely recognition of these syndromes by flagging pertinent findings, discussing these in the multi-disciplinary team setting and implementing the appropriate genetic testing.

P39

Ex Vivo Fluorescence Confocal Microscopy Analysis of Basal Cell Carcinomas in Mohs Surgery

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Ex vivo fluorescence confocal microscopy (FCM) allows rapid real-time analysis of freshly excised tissue. We designed a prospective cohort study aimed at determining the sensitivity and specificity of ex vivo FCM imaging in detecting residual basal cell carcinoma (BCC) in tissue freshly excised during Mohs micrographic surgery. The time taken for this analysis was compared to standard Mohs frozen tissue pathology analysis in our laboratory. The 4th generation Vivascope 2500 (released in 2018) with 488nm and 785nm lasers can simultaneously scan tissue dipped in acetic acid and acridine orange to produce very similar digital images to conventional haematoxylin and eosin staining. Importantly, the fresh tissue can still undergo conventional histological preparation after FCM with no damage to the tissue or retention of dyes used. Thirty-three patients met the criteria. Their fresh tissue samples were analysed by FCM by the bedside as well as formal review of frozen tissue analysis as in conventional Mohs. One of the main drawbacks in FCM analysis, as it is sometimes with frozen tissue, was the tissue could not be flattened adequately to enable us to accurately view the peripheral margins. However, with the deep margin, specificity was 100% and sensitivity 80%. What is outstanding and noted in this study was the clarity of FCM and how the images are incredibly similar and also much faster (at least 16 minutes) than analysis by frozen section. With a new flattening device, it should become a real game changer for Mohs surgery.

P40

The Microbiome can be Analysed from Simulated NHS Bowel Cancer Screening Programme Faecal Immunochemical Test Samples

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In work presented at the previous Pathological Society meeting, we demonstrated that microbiome analysis of NHS Bowel Cancer Screening Programme (NHSBCSP) guaiac Faecal Occult Blood Test (gFOBT) samples improved colorectal cancer (CRC) screening accuracy. As the NHSBCSP is transitioning from gFOBT to the Faecal Immunochemical Test (FIT), we assessed whether microbiome analysis could be performed using FIT. A small number of technical studies had indicated this might be possible, although none had replicated NHSBCSP conditions. Two healthy volunteers provided two stool samples. Stool was manually homogenised and the following were made in triplicate: gFOBT stored at room temperature, whole stool aliquots stored at -80°C, and FIT. FIT were stored for 3 days at room temperature (to simulate postage to the screening Hub); the foil was then pierced and liquid squeezed into the chamber (to simulate Hub processing); FIT were then sealed and stored for 5 days at either room temperature, 4°C or -80°C (to simulate transfer to the laboratory). DNA extraction was performed on day 8. V4 16SrRNA sequencing was performed. DNA extraction and sequencing was successfully performed for all samples, in contrast to one study which reported difficulty extracting DNA from FIT. Bray Curtis distances between a reference whole stool aliquot and replicates were lower than between the reference and FIT or gFOBT; however there was no difference in Bray Curtis distances between the reference and FIT (stored under different conditions) or the reference and gFOBT. There was no difference in the relative abundance of CRC-associated taxa between types of sample or storage conditions beyond that due to subsampling. These results indicate that the microbiome can be analysed from simulated NHSBCSP FIT samples with results equivalent to gFOBT. This study informs the design of a definitive study to investigate whether microbiome analysis of NHSBCSP FIT samples improves CRC screening accuracy.

P41

Ex Vivo Fluorescence Confocal Microscopy (Ex-FCM): Can it Replace Routine Processing of Formalin-Fixed Prostatic Core Biopsies to enable Rapid Diagnosis?

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Background: The potential benefits of Ex-FCM in replacing frozen sections are well reported [Longo C et al, Br J Dermatol:2018, doi.org/10.1111/bjd.17507; Puliatti S et al, BJU Int., 2019, doi:10.1111/bju.14754]. We investigated if similar benefits could be demonstrated in formalin-fixed tissues.
Methods: 83 formalin-fixed samples of prostatic tissue from 9 patients were washed in saline (30s), IMS 95% (20s), Acridine Orange (30s) and finally acetic acid (20s) and then imaged (Mavig Vivascope 2500G4). Uniformity of focus, completeness of scanning, staining of all cellular components, clarity of nuclear details and similarity to routine H&Es were scored on the images as 1-present or 0-absent. A total score out of 5 was calculated. The Ex-FCM diagnoses were compared to the original results. 9 samples were immunostained (IHC) variously for CK5/6, AE1/3, MNF116 and PSA and compared to conventional samples.
Results: The cases scored 0 (n=1), 1 (n=3), 2 (n=15), 3 (n=64); no cases scored 4/5. The Ex-FCM diagnoses were benign (68) and adenocarcinoma (12); all were correct. The remaining 3 (3.6%) samples were uncertain, originally diagnosed as benign (2) and ASAP (1); these were excluded. Sensitivity and Specificity were both 100%. Quality of IHC staining on the Ex-FCM samples equalled the conventional samples.
Conclusions: The results are promising - there may be a role for the EX-FCM in the diagnostic process of formalin-fixed prostatic biopsies, and possibly other tissues too. The Ex-FCM saves time taking less than 3 minutes from procedure to diagnosis and reduces the need to use the harmful reagents in conventional processing. Quality of IHC staining is not altered. It would help to alleviate shortages in BMS staff. The Ex-FCM images pose different diagnostic challenges, and therefore current diagnostic criteria may have to be revisited. Improvements to the image quality, in particular to nuclear detail, are still needed for confocal images to completely mirror H&E.

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Does Ex Vivo Fluorescence Confocal Microscopy (Ex-FCM) Interfere with Immunohistochemistry (IHC) Staining?

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Background: Ex-FCM is an imaging technique that allows examination of fresh skin tissue. It eliminates the need for conventional tissue processing. Cinotti et al [Dermatology Practical and Conceptual, 8(2), pp.109-119; 2018] had shown that when the Ex-FCM is used on fresh tissue it does not interfere with the quality of subsequent formalin-fixed paraffin sections produced using conventional methods. The results of our concurrent study of using Ex-FCM in formalin fixed tissue are promising. We aim to determine whether or not Ex-FCM on formalin fixed tissue would interfere with IHC result.
Methods: 28 formalin-fixed samples (23 prostate biopsies and 5 breast biopsies) from 8 patients were included in this study. Of the 28 samples, 15 were Ex-FCM-imaged followed by conventional processing and 13 went through conventional processing only. The Ex-FCM samples were washed initially in saline (30s) followed by IMS 95% (20s), Acridine Orange (30s) and finally acetic acid (20s) before scanned using the Mavig Vivascope 2500G4. A panel of antibodies (CK5/6, AE1/3, MNF116, PSA, ER, PR and HER-2) were used on paraffin embedded sections. Specificity, intensity and uniformity of staining were assessed and a score of 1=present or 0=absent was assigned; a total score out of 3 was calculated. Finally, we analysed the results of the 2 sample groups.
Results: Total number of IHC tests was 41 (CK5/6=23, AE1/3=5, MNF116=5, PSA=2, ER=2, PR=2, HER-2=2), as some samples had > 1 antibody tested. Of the 41 tested antibodies, 29 were on prostatic and 12 on breast tissue. All breast and prostate samples from both the Ex-FCM and conventional groups scored "1" for specificity, intensity and uniformity of staining. All sample in both groups showed 100% identical staining.
Conclusion: This pilot study shows that the Ex-FCM technique does not interfere with IHC staining and that the quality of IHC staining on both sample groups is similar. A larger study testing a wider spectrum of antibodies remains merited.

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Pardus: An Affordable Open Source Hardware and Software Robotic Platform for Standard Microscopes

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Purpose: To develop an affordable open source platform to automate a routine microscope. There are many applications (e.g. live cell imaging, 3D deconvolution) for which specialist digital pathology and whole slide imaging (WSI) scanners are not optimal or applicable. Also, WSI scanners are prohibitively expensive for people without access to huge budgets or central resources. While there is much open source software, open source hardware has, until now, been largely dedicated to 're-imaginings' of the microscope itself rather than automating routine diagnostic pathology scopes.
Methods: Pardus™ (Programmable, Affordable, Remote-controlled Drive Utility Standard) is an open source hardware and software combination I developed to meet this need. Pardus allows a standard microscope to be motorised to 3 (or 4, i.e. objective turret) axes with inexpensive off-the-shelf components (Nema17 geared stepper motors, A4988 drivers controlled from a Raspberry Pi (RPi) computer) and some custom 3D printed parts. Use of the RPi makes the scope controllable via WiFi, Bluetooth (e.g.via smart phone) and by internet browser from any location. The system implements bi-directional communication to an (optional) separate image processing computer via the third party ZeroMQ library (also open source). The scope can be fully programmed in C (and other languages) as well as with a custom script language that requires no knowledge of formal computer programming.
Results: Results from Pardus fitted to a 1975 Zeiss Standard microscope show a high degree of stage repositioning accuracy (upto <5 microns in X,Y). This system performed effective automated digital image capture, multi-frame averaging, background correction, autofocussing, tissue detection and slide scanning as well as a machine vision task and 3D deconvolution.
Conclusions: Pardus allows the use of standard microscopes for slide scanning, machine vision and other automated / remote microscopy tasks as an affordable open source solution.

P44

IgG4 Related Disease: An Analysis of the Clinicopathological Spectrum

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Background: IgG4 related disease (IgG4RD) is a fibro inflammatory multisystem disease diagnosed when increased IgG4 positive plasma cells>10cells/ hpf, thrombophlebitis, storiform fibrosis and IgG4/IgG ratio > 40% are present (Boston criteria).This is a retrospective study analysing the clinicopathological features in patients with suspicion of IgG4RD.
Material and methods: Winpath histology database was searched over a period of 7 years (Jan 2011 to April 2018) to identify all cases of suspected IgG4RD wherein IgG4 immunohistochemistry was performed. The histology slides were reviewed to categorize cases into Boston criteria groups – highly suggestive of IgG4RD, probable IgG4RD and insufficient evidence. Information regarding clinical data, treatment received, follow up and serum IgG4 levels was obtained from medical records and APAS clinical database.
Results: The study included 204 patients and the most common sites of biopsy/ resection were pancreas and duodenum. The most common clinical presentation was fibroinflammtory lesion or mass/ lump. On histology, 54/ 204 (26.47%) cases showed storiform fibrosis, 65/ 204 (32.64%) had >10 IgG4+ plasma cells per high power field and only one case showed thrombophlebitis (0.49%). 14/204 (6.78%) cases were categorized as highly suggestive of IgG4 RD; 8 of these showed high serum IgG4 levels and were managed clinically as true IgG4RD. 13/204 (6.35%) were categorized as probable IgG4RD with 6 showing raised serum IgG4 levels and treated as IgG4RD while 177 (86.76%) had insufficient evidence. Only 16 cases showed IgG4/ IgG ratio >40%.
Conclusion: Histological diagnosis of IgG4RD is difficult as all characteristic features are not always present especially in small biopsies. Due to novelty of its experience, fear of over diagnosis in the context of malignancy and features overlapping with diseases of similar clinical scenario, diagnosis of IgG4RD has become more challenging. Further multicentre clinical trials/ studies are advisable.

P45 *

Schistosomiasis Education Programme Developed for Children and Healthcare Leaders in the Marolambo District, Madagascar

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Purpose: Schistosomiasis is a significant public health concern in sub-Saharan Africa. In Madagascar, schistosomiasis is endemic in 107 of 114 districts, and in the Marolambo district of Madagascar, 94% of school aged children (SAC) were infected in a 2015 study. Access to safe water, sanitation and hygiene plays a key-role in disease transmission. Improving health education and understanding of schistosomiasis can contribute towards reducing disease transmission and subsequently disease prevalence. With the help of a Path Soc Grant Madagascar Medical Expeditions (MadEx) visited rural Madagascar to promote a sustainable schistosomiasis education programme by means of a programme delivered to teachers, villages leaders and medical professionals. Additional aims of the project involved undertaking schistosomiasis morbidity research and providing treatment.
Methods: An education programme was designed to be low cost and sustainable. This included a mixture of interactive games, songs, posters, and lessons delivered to communities living in six villages along the Nosivolo river, Marolambo. Knowledge, attitude and practice (KAP) questionnaires were carried out on 389 randomly selected SAC pre and post education to assess their understanding of the disease and impact of education.
Summary: Post education questionnaires showed consistent improvements in knowledge across all themes assessed. Specifically, SAC were able to identify water contact as a transmission mode (75% to 93%), showing accurate understanding of symptoms (31% to 70%) and awareness of antihelminthic drug Praziquantel as treatment (52% to 92%).
Conclusion: With thanks to Path Soc we were able to highlight the effectiveness of education activities, which are low cost and feasibly delivered in a remote, rural setting on a high-risk population. Through educating children, teachers and medical professionals this will hopefully result in risk-averse behaviour, reduce disease transmission and overall prevalence.

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P46

The Evolutionary Dynamics of Chromosomal Instability and Genome Doubling in Colorectal Cancer

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Purpose of the study: Chromosome copy number alterations are ubiquitous features of carcinogenesis. It has been hypothesised that colorectal cancer (CRC) development, in particular, involves gains and losses of specific chromosome arms. Further, it is thought that these events tend to occur in a preferential order. The Genomics England 100K Genomes Project has provided an unprecedented opportunity to test these hypotheses by inferring the evolutionary dynamics of mutational events across hundreds of CRC genomes.
Methods: We processed, quality validated, and called chromosome copy number alterations across over 1800 whole genomes from the latest data release of the 100K Genomes project. Using this data, we performed a molecular clock analysis, based on a Bayesian statistical framework, of passenger mutations within copy events, which serves to order chromosome copy number aberrations relative to patient age at cancer resection.
Summary of results: We found that a some mutational order does exist within CRCs, namely that 5q loss tends to occur prior to other chromosomal aberrations. However, we also show evidence that many cancers evolve through a 'punctuated' accrual of mutations, rather than a 'gradual' accumulation as classically depicted. Punctuated evolution might represent the occurrence of either 'chromosomal catastrophes' or genome doubling. Further, we also demonstrate that cancers have "long" or "short" time intervals elapsed between 5q loss (likely the initiating event that causes premalignant adenoma growth) and the time of operation (when late stage cancer was resected).
Conclusions: Mutational orders are quite variable in CRC. We speculate that the observed evolutionary 'jumps' across karyotype space are responsible for initiating malignancy. Our analysis demonstrates how whole genome sequencing data can provide new insight into the complex evolutionary dynamics of chromosomal instability in solid tumours.

P47

Tumour Infiltrating Lymphocytes Assessed on Haematoxylin Eosin Stained Pre-Treatment Biopsies of Oesophageal Cancer: Evaluating Interobserver Variability

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Objective: To evaluate interobserver variability (InterVar) in assessing tumour infiltrating lymphocytes (TILs) in pre-treatment endoscopic biopsy pieces (BPs) from patients with oesophageal cancer (OeC) recruited into the OE02 trial.
Methods: Haematoxylin Eosin (HE) stained slides from 938 BPs from 305 patients (average 3 BPs/patient, range 1-12) were reviewed by 2 observers. The %area TILs in stroma (sTILs) and tumour cell compartment (tuTILs) was first scored independently as 0%, 5%, 10%, 20% ... 100% or unclassifiable following international guidelines. Disagreement was defined as any absolute score difference between observers greater than 10. Second, discordant scores were jointly reviewed and scored. Reasons for unclassifiability were recorded. InterVar was analysed using all categories or median dichotomised data.
Results: In total, BPs from 285 (93%) OE02 trial patients were classifiable. The most frequent causes of unclassifiability were uncertainty of precise TILs location, artefacts, small BP size, low stroma or tumour content per BP or morphological similarity of single tumour cells and TILs. The initial interobserver agreement was 80% and 79% for tuTILs and sTILs, respectively, using all categories and 80% and 81% for tuTILs and sTILs, respectively, using dichotomised data. This level of agreement corresponds to κ values of 0.7. After joint assessment, complete agreement for all scores for tuTILs and sTILs was achieved.
Conclusions: This is the first study to demonstrate that the immune response can be assessed reproducibly in the stroma and tumour cell compartment on routine HE stained BPs from OeC patients. Our study suggests that some endoscopic biopsies might be unclassifiable emphasising the need for a sufficient number of high quality biopsies. Studies are now underway to investigate the clinical value of TIL assessment in the pre-treatment biopsy of OEC patients.

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Tumour Infiltrating Lymphocytes Assessed on Haematoxylin Eosin Stained Pre-Treatment Biopsies of Oesophageal Cancer Patients Predict Benefit from Chemotherapy: Results from the MRC OE02 Trial

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Introduction: Neoadjuvant chemotherapy (NAC) followed by surgery is one standard of care for treatment of patients with locally advanced (resectable) OeC. However, patient outcomes remain poor. It has been suggested that only a subset of patients might benefit from NAC. Studies in breast cancer suggest that tumour infiltration by lymphocytes (TILs) can predict benefit from NAC. We explored whether TILs can predict benefit from NAC in OeC patients recruited into the OE02 trial.
Methods: Haematoxylin/Eosin (HE) stained sections from the pre-treatment biopsy were collected retrospectively from 158 patients treated with surgery alone (S patients) and 147 patients treated with NAC + surgery (CS patients). Slides were analysed by 2 independent observers quantifying %area TILs in the stroma (sTILs) and tumour cell compartment (tuTILs) separately according to international guidelines. The relationship of the lowest, highest and average TILs score per patient and compartment with clinicopathological parameters, survival and treatment interaction was investigated.
Results: Linear treatment interaction was observed for the lowest tuTILs score (interaction p=0.0236). The median (range) of the lowest tuTILs was 0% (0-20%). CS patients with a lowest tuTILs of ≤5% (n=122) had a survival benefit compared to S patients (n=123), p=0.012. Survival of CS patients with a lowest tuTILs >5% was similar to that of S patients. There was no relationship between lowest tuTILs score, histological phenotype, gender, or age. All sTILs scores, highest or average tuTILs scores showed no treatment interaction. All OeC were EBV negative and MMR proficient.
Conclusion: This is the first study suggesting TILs measured on HE in the tumour compartment of pre-treatment biopsies from OeC patients can identify patients who benefit from NAC. Our findings represent an important step forward towards personalising treatment for OeC patients. Validation in a second independent cohort is underway.

P49

Single Regulatory T cell Based Theranostics in Colorectal Cancers (CRCs)

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DEPArrayT is one of the best automated platforms that can identify and recover target cells with high resolution and purity required for sensitive downstream genomic and expression analyses (Nature Med, 2017). Here, we adopted a state-of-the-art multi-modal strategy including the real-time imaging-based, single-cell sorting DEPArrayT with downstream molecular assays, and multiplex immunohistochemistry/immunofluorescent technique (mIHC/IF, Pathology, 2018) to diagnose disease as well as predict clinical outcome in order to direct therapy, based on immune cells.
Proof-of-concept study was conducted by examining blood circulating and tumor-infiltrating regulatory T cells (Tregs) from patients with colorectal cancers (CRCs) with Caucasian population (Immunity, 2016). We then further expanded to a larger Asian patient cohort in Singapore, focusing on some of the markers including OX-40 and PD-L1. Our results demonstrated that DEPArrayT enables automated discrimination and isolation of a rare signature Tregs population which expressed phenotypically distinct surface markers and several differential expressed genes. Statistical classification analysis suggested that blood circulating signature Tregs could be potentially used as diagnostic biomarker to predict clinical outcome of CRC patients which decently achieve 75% for both specificity and sensitivity. mIHC/IF staining of tissues from 264 CRC patients further validated this finding that the higher density of these signature Tregs is associated with better disease-free and overall survival of CRC patients.
This study provided comprehensive characterizations of CRC-specific signature Tregs at single cell resolution, and could provide a paradigm shift to revolutionize single-cell based precision diagnostics so as to prevent mistreatment, and to reduce morbidity and mortality associated with devastating diseases. Ongoing studies aim to validate the finding in a larger and multi-centered cohort.

P50

Tumour Deposits as an Independent Prognostic Factor for Colorectal Cancer

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Background: The presence of tumour deposits (TD) is a prognostic indicator for Colorectal cancer patients, although there is limited research into the extent which TD can be relied on as a prognostic indicator in patients with Lymph Node metastasis. Current staging systems acknowledge TD presence only in the absence of lymph node metastasis. Further analysis of patient outcomes based on the newest TD definitions as published in the 8th edition TNM Classification is needed to assess TD impact, independent of lymph node metastasis. This study investigates the significance of TD as an independent prognostic factor, the clinicopathological factors linked to TD incidence and assess Recurrence Free Survival (RFS) of colorectal cancer patients with TD.
Method: A single centre study of 233 patients with stage II/III colorectal cancer (all underwent surgery between January 2009 and December 2011) were assessed for TD and lymph node metastasis using histopathology slides stained with Haemotoxylin and Eosin. TD status is compared against RFS, obtained from follow-up appointments after surgery.
Results: 25% of patients had positive TD status. Left sided and rectal tumours, lymphatic invasion and lymph node metastasis were associated with TD incidence. RFS for both stage II and III patients with TD positive status were significantly lower than the same staging groups without TD. Patients with TD presence had significantly worse RFS irrespective of any lymph node metastasis.
Conclusion: TD are a significant prognostic indicator for stage II/III colorectal cancer patients, independent of lymph node metastasis. The ability of TD to separate the two groups of mixed stage II and III patients in this study infers the tumour deposit status of the patients may provide more accurate prognostic information than lymph node metastasis. We propose revision of the AJCC TNM staging system to include recording TD as part of cancer staging, in the presence of lymph node metastasis.

P51

Deep Learning Algorithm for Colorectal Cancer Detection

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Background: Colorectal cancer (CRC) is the second largest cause of cancer mortality in the UK. With early diagnosis and appropriate treatment, 57% of patient can survive for 10+ years. Both diagnoses and treatment decisions are made manually on glass slides or digital slides. Manual analysis of tissues requires visual inspection of highly complex cellular structures, which is time consuming, subjective and prone to error. Deep Learning (DL) has the potential to automate this task, improving on speed, objectivity and accuracy. Current research at the University of Leeds uses DL algorithms to classify CRC tissue in order to automatically predict response to therapy. However, it requires cancer tissue to be annotated by a pathologist prior to analysis which has the same disadvantages. This project aims to develop a DL algorithm to detect CRC on digital slides, as a pre-processing step for downstream image analysis.
Method: 11,977 images from UMM and NCT tissue banks manually annotated as either tumour (colorectal cancer and stomach cancer epithelial tissue), stroma and muscle or adipose and mucus were used to train the Resnet18 network. The model is trained with 5-fold cross validation to identify aforementioned 3 categories. A heatmap is generated to representing probability of cancer cells on a whole-slide image and compared with existing pathologist annotations. Jaccard and Dice similarity, detection accuracy and tumour to stromal ratio(TSR) will be used to assess efficacy.
Results: The model attained validation accuracy of with an AUC of lowest 0.989. The preliminary tumour prediction reached a Jaccard similarity, Dice similarity and tumour detection accuracy of 0.65, 0.79, 0.90 respectively. TSR from algorithm is 0.93 different from 0.58 from pathologist which needs further review.
Conclusion: Robust automatic detection of colorectal cancer will allow for higher throughput of patient samples, allowing pathologists to make more comprehensive treatment decision.

P52 ✱

Exploring the Role of Light-Sheet Microscopy in Improving the 3D Visualization of Early Colorectal Cancer (pT1/pT2)

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Backrgound: Each year 1.8 million people are diagnosed with colorectal cancer worldwide. Current treatment options include surgery, chemotherapy, radiotherapy and immunotherapy. There are many potential complications of major surgical resection for this disease therefore we should move to local excision where possible. To do so, better methods of predicting lymph node metastases are required to avoid leaving behind residual disease.
Methods: Tissue was collected after tumours had been pathologically staged. Antibodies to blood vessels, lymphatic vasculature and epithelial cells were optimised through immunohistochemistry, immunofluorescence and confocal microscopy in 2D before transferring onto 3D tissue samples to be optically cleared for light-sheet microscopy. Lycopersicon Esculentum lectin was also trialled for immunofluorescent staining of blood vessels. Data from the light-sheet microscope was analysed using Imaris software. Reverse clearing techniques were trialled.
Results: Epithelial and lymphatic vasculature staining proved unsatisfactory using light-sheet microscopy. Specific staining of blood vessels was captured in 3D using antibody and lectin staining. Use of a pre-conjugated lectin provided specific staining with little background staining while also reducing sample preparation time by over 60%. Reverse clearing provided evidence that samples used for light-sheet microscopy can subsequently be used in routine diagnostic techniques. This project has provided 3D images of blood vasculature within the bowel wall with evidence that dual staining of 3D tissue may be possible in the future. It has also demonstrated that tissue which undertakes optical clearing and light-sheet microscopy can undergo current diagnostic procedures after optical clearing has been reversed. Therefore, light-sheet microscopy could become a primary diagnostic modality where 3D structure was critical to such assessments.
✱ ***This project was kindly funded by the Pathological Society of Great Britain & Ireland.***

P53

Immune Status is Prognostic for Poor Survival in Colorectal Cancer Patients and is Associated with Tumour Hypoxia

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Purpose of the study: CD3/CD8 by immunohistochemistry is a prognosticator for colorectal cancer. Here we evaluate the suitability of alternative immune classifiers on prognosis and determine related molecular biology which may be amenable to targeted-therapy.
Methods: The prognostic potential of immune biomarker combinations relating to CD3/CD8 were evaluated using digital image analysis platform QuPath and compared using cox regression for overall survival on a series of independent cohorts. Patients were assessed for immune (CD3, CD4, CD8, CD20, FOXP3) and immune checkpoint (ICOS, IDO-1, PD-L1) biomarkers. Matched mutational and transcriptomic data were interrogated to identify associated biology.
Summary of results: In 1750 stage II-IV primary colorectal cancer patients, 1449 (82.80%) had complete immune biomarker data, CD3/CD4/CD8 status was found to be an independent predictor for overall survival in patients with colorectal cancer. Specifically, inclusion of CD4-positive lymphocytes to CD3/CD8 scores improved prognostic power across all stages of colorectal cancer, particularly in patients with stage IV disease (HR 1.98 [95%CI: 1.47-2.67] vs HR 1.31 [95%CI: 1.02-1.67] for CD3/CD4/CD8 and CD3/CD8 respectively). CD3/CD4/CD8-low patients were found to associate with transcriptomic tumour hypoxia, confirmed using CAIX IHC (p=0.0009), which may mediate disease progression through common biology (KRAS mutations, CRIS-B subtype, and SPP1 overexpression).
Conclusions: Given the significantly poorer survival of CD3/CD4/CD8-low patients, these data provide compelling evidence for use of CD4 to complement low CD3/CD8 scores to predict patient prognosis in colorectal cancer and potentially may facilitate immunophenotyping on patient biopsies. Additionally, we have associated our CD3/CD4/CD8-low patients with a difficult-to-treat, poor prognosis hypoxia signature; these patients may derive benefit from inclusion on hypoxia-targeting clinical trials.

P54

Profiling Uncoupling Protein 2 in Colorectal Cancer

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The incidence of colorectal cancer remains high and there is a clear need to identify novel biomarkers of this type of tumour. In a previous study, we showed that the expression of uncoupling protein 1 (UCP1) was associated with prognosis in colorectal cancer. Therefore, the aims of this study were to develop a monoclonal antibody specific to uncoupling protein 2 (UCP2) and to characterise its expression in colorectal cancer. A monoclonal antibody to UCP2 was developed using a short peptide immunogen which was selected through comprehensive analysis of the structural and physicochemical properties of the UCP2 protein. The antibody was validated by immunohistochemistry, immunoblot and mass spectrometry analysis. The antibody was then used to evaluate the expression of UCP2 by immunohistochemistry in a tissue microarray containing 823 primary colorectal cancers. The immunostaining of tissue cores was scored using both a manual semi-quantitative system and by image analysis using QuPath software. UCP2 showed cytoplasmic localisation and there was a significant agreement between the two scoring methods (κ=0.60, p=<0.001). The results showed the expression of UCP2 was significantly higher in primary colorectal cancer compared to normal colonic epithelium (p<0.001). The expression of UCP2 was significantly associated with lymph node stage (p=0.002) and Dukes stage (p=0.002). Furthermore, high expression of UCP2 was significantly associated with worse survival using a cut-off point determined by ROC analysis (HR=1.291, 95%CI=1.032-1.614, χ^2=5.067, p=0.024) and a cut-off point based on quartile dichotomisation (HR=1.330, 95%CI=1.044-1.696, χ^2=5.385, p=0.020).
In conclusion, a monoclonal antibody to UCP2 has been successfully developed and that UCP2 is overexpressed in colorectal cancer with high expression being associated with worse survival.

P55

Tumour Buddin in Pre-Diagnostic Cancer Biopsy Samples Comparing Haematoxylin and Eosin with Cytokeratin Staining

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Conventionally TB in biopsies (intra-tumoural budding/ITB) is assessed on haematoxylin and eosin (H&E) staining. However the use of cytokeratin (CK) immunohistochemical staining has been shown to improve both the quantity of TB identified and interobsever variability. We aimed to assess CK and H&E in the assessment of TB in small biopsy samples in a large cohort of neoadjuvant chemoradiotherapy patients with rectal cancer. 184 rectal cancer pre-therapy biopsy cases were selected from our colorectal cancer database. Each biopsy had to contain at least one complete/full face of invasive carcinoma at 200x magnification in at least one tissue fragment for inclusion. The biopsies were assessed for ITB on both haematoxylin& eosin and cytokeratin slides using a 20x objective/per eye piece diameter of 0.785mm^2. Assessable field number and tissue fragment number per biopsy were also noted. 101 (75%) of the biopsies were positive for ITB using CK compared to 47 (25%) using H&E. Median ITB/biopsy for CK was 3.2 compared to 1.3 for H&E. While a total of 1057 tissues fragments were present between the 184 biopsies, only 593 (56%) contained invasive cancer, a pre-requisite for the assessment of TB.
This study demonstrates that ITB can be assessed manually on small biopsy samples (in preparation for digital analysis), and that the use of CK greatly improves ITB assessment rate. It also demonstrates the importance of assessable field number over tissue fragment number. The predictive power of biopsy ITB (on H&E or CK) will need significantly larger numbers to assess its potential in a clinical setting.

P56

Carcinosarcoma of Common Bile Duct: A Case Report

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Backgound: Carcinosarcomas are rare malignant biphasic tumours with both epithelial (carcinomatous) and mesenchymal (sarcomatous) elements. They can arise in any organ, the uterus being the most common location. However, carcinosarcoma of the gallbladder or bile duct has been infrequently reported.
Case report: A 59-year-old man presented with obstructive jaundice. CT scans showed a localised mid-common bile duct stricture and cytological brushings were consistent with an underlying carcinoma. Frozen section assessment of lymph nodes followed by resection of the extrahepatic biliary tree with gall bladder was performed. Histopathological examination confirmed the diagnosis of carcinosarcoma of common bile duct with retropancreatic lymph node metastasis. A month later the patient developed multiple liver metastases. Unfortunately, the patient rapidly deteriorated and died post-surgery, before having had any post-operative adjuvant chemotherapy.
Conclusion: Pre-operative definitive diagnosis of carcinosarcoma is challenging owing to vague clinical presentations, non-specific radiological features and an anatomical location that is relatively inaccessible for biopsy and cytological studies. The use of frozen section presents difficulties, which we have examined in our case presentation. The prognosis is notoriously poor with a median survival of 5.5 months. Owing to the rarity of the tumour incidence and poor prognosis there is limited experience in diagnosing and managing such cases and a standardized approach to treatment is yet to be developed.

P57 *

Investigating the Stem Cell Dynamics of Gastric Intestinal Metaplasia In Vivo

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Purpose of study: There is an urgent need to develop spatiotemporal models of early cancer progression through quantifying the in vivo stem cell dynamics of precursor lesions. A detailed understanding of stem cell evolution of intestinal metaplasia may facilitate gastric cancer risk stratification and targeted intervention.
Methods: 3µm serial sections of gastric mucosa were cut from gastric sleeve bariatric resection specimens, which were embedded en face. The tissue was labelled with immunohistochemistry for the transcription factor CDX-2 - a specific marker of the intestinal lineage. Serial sections were scanned and analysed using the digital pathology software QuPath. Analysis centred on gastric glands that were partially metaplastic, calculating the proportion of CDX-2 positive nuclei for each partially metaplastic gland, and the change in proportion through successive levels. This was used as a proxy measure of competition and the fitness of the metaplastic phenotypic change.
Summary of results: Partially metaplastic glands were rare in bariatric resection specimens (mean frequency 2.54 glands x10-4 (range 2.21-3.12 x10-4)). The results from eight partially metaplastic gastric glands indicate that metaplasia achieves fixation through neutral drift, with a mean change in clone size between sections of 0.21 (p value = 0.09). This indicates that metaplastic clones do not have a competitive advantage over their neighbouring lineages, even in the setting of chronic inflammation. 3D reconstruction of the origin of metaplastic clones suggests that each tubule of a gastric gland is an independent stem cell unit. Finally, we find that metaplastic clones uniquely derived from the isthmus region of the gastric oxyntic gland, not from the basal region.
Conclusion: This study provides quantitative data on how the metaplastic phenotype achieves clonal fixation and may clarify the location of the stem cell zone in human gastric oxyntic glands.
* ***This work was performed as a Summer Student in UCL Research Department of Pathology and was funded by Prof AM Flanagan and the Pathological Society of Great Britain & Ireland.***

P58

This abstract has been withdrawn

P59

Ten Year Audit of Common Bile Duct Brushing Cytology: Outcomes in a National Hepatobiliary Unit

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Aims: To correlate Common Bile Duct (CBD) brushing cytology reporting in a large National Practice with relevant surgical outcomes.
Materials and methods: 1272 patients with at least one biliary brushing, were identified from the laboratory information system in the period 1.1.09 to 31.12.18. 413 relevant surgical specimens that met inclusion criteria were identified. Sensitivity, specificity, PPV and NPV were calculated: once with malignant cytology alone and the other with malignant and suspicious cytology, counted together as malignant for analysis.
Results: 6 of 1272 cases were identified as false positives. Three false positive cases were diagnosed as malignant and three as suspicious. The Sens, Spec, PPV and NPV were 0.48, 0.87,0. 98 and 0.09 respectively. If malignant and suspicious were analysed as positive the respective figures were 0.64, 0.74, 0.98, 0.11. The high PPV with a relatively low specificity is related to the low number of true negative surgeries (20 or 17 respectively) in the cohort. Three of the false positives were IgG4 disease and we have not had such a case since 2014 as diagnostic features clinically, radiologically and cytologically became firmer.
Conclusion: CBD brushing is an accurate diagnostic modality, as demonstrated here in a very large cohort of cases. There has been a reduction in the number of false positive diagnoses in the past 5 years due to increased ability to identify autoimmune pancreatitis.

P60

An Unusual Pancreatic Neoplasm: A Case Report

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Introduction: The most common pancreatic tumour is a primary ductal adenocarcinoma (85 - 90%). Other less common tumours include solid pseudo-papillary tumours and acinar cell carcinoma with rare instances of metastatic tumours (1-2%). Metastatic disease can occasionally present as an isolated mass in the pancreas.
Case report: A 74 year old female presented with obstructive jaundice. She was diagnosed with a 4x3.8 cm pancreatic mass on CT scan and was treated with Whipple's surgery. Histopathology showed a 41mm atypical small round blue cell tumour in the posterior pancreas arranged in sheets with fibrous septae and rosettes. There were areas of multinucleate giant cell change with occasional rhabdomyoblasts. The mitotic count was 7-10/hpf with areas of necrosis and apoptosis. On immunohistochemistry the tumour was positive for myogenin, desmin, CD10, CD56 and SMA. The proliferation index with MIB-1 was 80%. Four lymph nodes along with the duodenal wall, pancreas and peri-pancreatic tissue were involved. The case was diagnosed as an alveolar rhabdomyosarcoma (RMS) most likely metastatic with an unknown primary. Unfortunately the patient deteriorated rapidly and passed away and the investigations to identify the primary site could not be performed.
Discussion: Tumours can metastasize to the pancreas with a single solid mass presentation. Alveolar RMS can metastasize to the pancreas although it is extremely rare and can be the first presentation. One needs to be aware of this scenario especially when the tumour appears to be poorly differentiated on histology.

P61

Distinct Macrophage Phenotypes are Associated with Location and Stage in Bladder Cancers

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Background: Bladder cancer (BC) have a distinctive high percentage of recurrence and progression into invasive stages. The lack of reliable predictive markers makes the disease management difficult, with regular invasive surveillance over patient's lifetime. Instillation of BCG gives excellent results in 60% of cases but the remaining patients either do not respond or suffer severe adverse effects. We hypothesise that both BC progression and response to BCG are influenced by the tumour immune environment (IE). Our purpose is to characterise the IE in BC to identify predictive markers for recurrence and response to BCG. We present here our results on macrophages (MP) phenotypes.
Methods: FFPE-biopsies (67 patients) representative of pathological stages and outcome; no previous treatment; 10 years follow up. Gene correlation networy analysis of published dataset GSE32894 (Graphia Professional) and Nanostring PanCancer IO360 Gene Expression Panel on our discovery set (n=22). Multiplex immunofluorescence (IF) (6 markers panel) on MP analysed with inForm software. High dimensional per cell data analysis and correlation with clinical data using SPADE and tSNE.
Results: Network analysis identifies 8 MP genes clusters that correlate with molecular subtypes but not grade or stage. One cluster seemingly defining tumour associated MP (CD163, CD14, ITGB2, AIF1, C1QA-B-C) is predominant in *highly infiltrated by non-tumour cells* (MS2b1) and *basal SCC-like* (MS2b2.2) subtypes. Nanostring confirms the lack of correlation with stage or grade and validates ITGB2, CD163, C1QA-B-C in our samples. Multiplex IF highlights the heterogeneity of MP with systematic differences between tumour-infiltrating and stromal MP. In pTa, the prevalent stroma phenotype is $CD68^{high}$, $HO1^{med}$, $MHCII^{low}$, $CD163^{low}$, $CCR2^{med}$, while almost 50% of infiltrating MP show high expression levels of those markers. In CIS 95% of infiltrating MP show a similar phenotype of activated MP with anti-inflammatory activity.

P62

Mixed Sex Cord Stromal Tumour of the Testis with Fibrothecoma and Sertoli Components: A Case Report

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Here we present a 46-year old patient with a mixed sex cord stromal tumour of the right testis, which is notable due to the presence of both fibrothecoma and Sertoli cell elements. The patient first presented with a two-week history of painless right scrotal swelling. Ultrasound imaging revealed a vascular rounded 3.5 x 3 x 3 cm heterogeneous solid lesion at the lower pole of the testis with a surrounding reactive hydrocele. Radical right inguinal orchidectomy was performed. Gross appearance of the tumour showed a firm whorled cut surface on sectioning with no abnormal appearance of the spermatic cord. Microscopically the tumour was well-circumscribed and pseudo-encapsulated with islands and cords of epithelioid and spindle-shaped cells separated by collagenous bands. The background stroma stained strongly positive for alpha smooth muscle actin while the epithelioid and spindle shaped cells stained positive for Melan-A, inhibin, and calretinin. Ki-67 immunohistochemistry was performed to assess mitotic activity and stained fewer than 1% of the lesional cells and no mitoses were seen, features which would be more supportive of a benign neoplasm. Although there have been individual case reports of patients with testicular fibrothecoma and mixed sex cord stromal tumours, to our best knowledge, there are no reports of patients with neoplasms exhibiting both a mixed fibrothecoma stroma and a Sertoli cell component. Due to the rarity of the presentation, tumour behaviour is difficult to predict, and it was advised that the patient remains under regular clinical review.

P63

Immune Profiling of the Tumour Microenvironment in Prostate Cancer Using Multiplex Immunofluorescence

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Purpose of the study: Prostate cancer (PCa) is known for its biological and clinical heterogeneity and identifying patients with an aggressive course is critical for providing radical interventions. Molecular profiling using immune cell phenotyping markers is vital to understanding the interactions of tumour infiltrating immune cells with malignant prostate epithelium. The aim of this study was to investigate the in situ phenotype, functional status and localisation of infiltrating immune cells in patients with PCa in order to determine whether they correlate with the ability of a tumour to metastasise to regional lymph nodes. We used a multiplex immunofluorescence (mIF), machine learning and automated quantitative scoring approach.
Methodology: A matched TMA was created from 94 patients who underwent radical prostatectomy with and without regional lymph node involvement. Two multiplex fluorescence panels for CD4, CD8, FoxP3, PD1 and CK and CD68, CD163, CD20 and CK were optimised and used for staining. We quantified the densities of those markers within the tumour epithelial and stromal compartments.
Results: Patients without lymph node metastasis had significantly more epithelial and stromal M1-like macrophages (p=0.046) and CD8 cytotoxic cells (p=0.001, p= 0.008). They were also richer in stromal effector CD4 cells (p=0.0003). The stromal CD4 effector immune cell density was a significant predictor of lymph node spread in the univariate and multivariate analysis. An independent TMA of 184 patients was also stained with the same mIF panels and the stromal CD4 effector cell density remained a significant independent predictor of nodal spread.
Conclusion: Together, these findings suggest differences in the immune infiltrate (particularly CD4 effector T cells) between PCa patients with vs without lymph node metastasis. This is important because it suggests further investigation is necessary into how immune microenvironment can affect clinical outcome.

P64

An Audit on MDTM Review of Prostate Core Biopsies

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Prostate cancer is the leading cause of cancer in men, causing 13% of male cancer deaths in 2016. The mortality rate is thankfully declining for prostate cancer. However, if we are to see further improvements in prostate cancer mortality statistics, an understanding of prostate cancer is vital to broaden our knowledge of the treatment options that are available.
The aims of this audit are to identify the level of variability between the reports, as well as adherence to the latest suggested guidelines from the Royal College of Pathologists, which outlines 5 core data items that should be included in a prostate core biopsy report. In this audit, the reports of prostate core biopsies done by Hospital A's histopathologists are compared to the reviews of Hospital B's histopathologists, reviewed for weekly Urology MDT meetings for network. A total of 201 cases were selected from MDTM lists between February 2013 to July 2018. Cohen's kappa statistics was used to assess the level of agreement between the two hospitals' reports.
The results informed that 2 out of the 5 core data items that should be included in a prostate core biopsy report had 'almost perfect' agreement at the 95% confidence level, with the remaining three having lower levels of agreement, especially for the core data item: 'Presence of Extra-Prostatic Extension', which had 'moderate' and 'slight' agreements between the pathologists. Majority of the reviews of these prostate core biopsies showed a high level of agreement. However, there were variations in the reporting of all the core data items, not helped by the lack of universal guidelines on reporting prostate core biopsies. Some degree of variability is expected in prostate core biopsies' reports as it is a highly subjective area, in particular grading and scoring of cancer. This further emphasises the need to review what degree of variability is acceptable without significantly affecting patient management.

P65

Heterogeneity of PTEN Loss in Prostate Cancer: A Study of Archival Radical Prostatectomy Specimens

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Background: Existing literature demonstrates links between losses of PTEN in prostate adenocarcinomas, and poorer patient outcomes. PTEN loss can be assessed by loss of protein expression on immunohistochemistry, loss by sequencing or a combination of both. In anticipation of potential use of this biomarker in clinical practice, in this study we aimed to assess whether PTEN expression across prostatic adenocarcinomas shows heterogeneity.
Methods: 5 archival formalin fixed paraffin embedded radical prostatectomy specimens of locally advanced prostate cancer were selected. 2- 4 regions to demonstrate anatomically interesting areas eg extraprostatic extension, seminal vesicle invasion, bladder neck invasion were selected plus a benign block from each case. Whole mount sections were stained for PTEN using automatic immunohistochemistry staining methods and targeted gene panel sequencing was undertaken.
Results: There was heterogeneous PTEN staining of all of the prostate tumours, with some tumours showing clearly distinct areas of loss and no loss as assessed by H Scoring. There was a trend to PTEN loss in more poorly differentiated areas. Furthermore, 2 of the 5 prostates sampled showed complete PTEN loss in all areas of the tumour sampled, and 2 others showed areas of complete PTEN loss in at least 1 sample. Only 1 did not show total PTEN loss in any area. Perineural invasion was interesting, showing areas of loss and no loss, often in the same tumour.
Conclusions: PTEN loss can be heterogeneous across a prostate cancer when multiple areas are examined from one tumour. This has implications for the use of PTEN loss as a biomarker of prognosis where biopsy material is relied upon, which only samples a portion of the tumour.

P66

An Unusual Variant of Uterine Carcinosarcoma

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Carcinosarcoma is a malignant biphasic tumour of poor prognosis comprised of high grade epithelial and mesenchymal elements. Specifically, these elements comprise carcinoma (usually endometrioid or serous) admixed with a homologous or heterologous mesenchymal component. Heterologous elements are most commonly rhabdomyosarcoma or chondrosarcoma. An unusual variant of uterine carcinosarcoma is presented here. A post-menopausal woman presented with PV bleeding. The resultant hysterectomy specimen showed a biphasic malignant tumour consisting of endometrioid adenocarcinoma admixed with a second component composed of pleomorphic epithelioid cells displaying prominent nucleoli and containing cytoplasmic pigment. The epithelial component was highlighted by cytokeratins and the second component of pleomorphic cells containing pigment stained positively for S100, Melan-A and HMB-45. On the resection specimen, the tumour invaded greater than 50% of the myometrium with lymphovascular invasion.
The findings in this case represent a uterine endometrioid adenocarcinoma exhibiting melanocytic differentiation, an unusual variant of uterine carcinosarcoma. A literature search shows that similar findings have been documented previously only once.

P67

A Rare Case of Intranodal Endometriosis Mimicking Metastatic Disease on Radiology

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Purpose of the study: Endometriosis is a common benign condition of reproductive age women which can occur at sites distant from the pelvis. Lymph node involvement by endometriosis, whilst documented, is a rare occurrence which is most often associated with grade 4, deeply infiltrating disease.
Methods: We describe a case of a 39 year old woman with a past medical history of grade 4 endometriosis who presented with abdominal pain. An MRI scan showed enlarged left external iliac lymph nodes and aortocaval nodes. Further characterisation with CT scan raised the suspicion of metastatic nodal disease in the absence of any radiologically detectable primary malignant tumour. Serum CA125 levels were raised at 330. All other tumour markers were normal.
Summary of results: The cut surface of the obturator lymph node showed a macroscopically visible cream/tan-coloured nodule. Histological assessment showed a large focus of decidualised endometrial stroma with endometrial glands and a strip of surface epithelium. The endometrial glands and surface epithelium were positive for AE1/3. The endometrial stromal cells were highlighted with strong positivity for ER, PR and patchy positivity for CD10. There was no histological evidence of a lymphoproliferative disorder or metastatic carcinoma. The pathogenesis of lymphatic dissemination in endometriosis is not well known, however differential expression of certain genes have been described in the literature.
Conclusions: Intranodal endometriotic deposits are a rare phenomenon and are not usually as large as demonstrated in our case. Herein we demonstrate a case of intranodal endometriosis of sufficient size to cause radiological suspicion.

P68 ✱

Evaluation of KCa3.1 as a Urinary Biomarker for the Monitoring of Disease Activity in IgA Nephropathy

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Background: IgA Nephropathy (IgAN) is the most common primary glomerulonephritis in the world. After diabetes, it is rapidly becoming a leading global cause of renal morbidity. 20-50% will develop ESRF within 10 years and at present, determining the likely course at diagnosis is difficult. Biopsy is the only definitive method to monitor progression. There is a clinical need for the identification of less-invasive methods. KCa3.1 is a calcium activated potassium channel shown to be involved in fibrosis. Work in our lab has shown synthesis of KCa3.1 varies in vitro in mesangial cells and proximal tubule epithelial cells (when stimulated by pathogenic IgA1) in a dose dependent manner. We hypothesise that KCa3.1 can be a useful marker, specific for IgAN disease activity in vivo.
Methods: The primary outcome was the presence or absence of KCa3.1 in urine samples. IgAN (n=50), disease (n=46) and healthy (n=28) samples were corrected for creatinine. Western blotting and densitometry were used to quantitate KCa3.1 profiles. Immunohistochemistry was used to identify renal origin of KCa3.1.
Results: KCa3.1 was found in all patients with IgAN (n=50), some disease controls (n=16) but no healthy controls. Presence was different in IgAN v DC ($p<0.0001$) and IgAN v HC ($p<0.0001$). The densitometry values also showed a difference between IgAN v DC ($p<0.00001$) and IgAN v HC ($p<0.00001$). Immunohistochemistry showed predominant staining within the proximal tubules. Densitometry values were not associated with proteinuria, haematuria or GFR. Performance measures found KCa3.1 was 100% sensitive and 65% specific.
Conclusion: KCa3.1 is not specific to IgAN thus limiting it's usefulness as a biomarker for disease. However, given existing evidence to suggest it's involvement in fibrogenesis with the consistent identification of KCa3.1 in all IgAN samples, KCa3.1 may be involved in IgAN fibrogenesis which may be a potential therapeutic target.
✱ ***Funded by Pathological Society of Great Britain & Ireland Intercalated Grant.***

P69

Primary Angiosarcoma Arising Within an Adrenocortical Adenoma: A Rare Case Report

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Introduction: Primary adrenal angiosarcoma and combined adrenal tumours are extremely rare neoplasms. We present a case of adrenal angiosarcoma arising within an adrenocortical adenoma, only four cases of which have been reported in the literature, to our knowledge.
Case report: A 49 year old male presented to the respiratory clinic with breathing difficulties, tiredness, reduced appetite and a sensation of a lump in the left lower chest. Imaging led to the discovery of an incidental 3cm left adrenal lesion. Blood tests showed subclinical hypercortisolaemia. Laparoscopic adrenalectomy was performed. Pathological examination revealed a well-circumscribed, pale brown and haemorrhagic adrenal nodule. Histologically the lesion had two distinct components: a peripheral zone resembling adrenocortical adenoma, and a central component comprising pleomorphic spindle and epithelioid cells associated with haemorrhage and exhibiting a high mitotic rate with atypical mitoses. Immunohistochemically the pleomorphic cells expressed CD31, CD141 and ERG, but not melan-A, inhibin, CD56, CD34, cytokeratins or S100 protein. A diagnosis of high grade angiosarcoma arising within an adrenocortical adenoma was made. Post-operative whole body nuclear medicine imaging revealed no other primary tumour site, nor evidence of recurrence or metastasis. At 10 month follow-up the patient was alive and well.
Discussion: Combined adrenal tumours are rare entities with uncertain pathogenesis. Most malignant adrenal lesions are metastatic in nature; primary malignancies, in particular angiosarcoma, are extremely rare. Primary adrenal angiosarcoma can present a diagnostic challenge for pathologists. It often exhibits epithelioid morphology and expresses cytokeratins, with potential for misdiagnosis as metastatic carcinoma or sarcomatoid adrenocortical carcinoma. The correct diagnosis can be made with an appropriately broad immunohistochemical panel. Treatment of choice is complete surgical removal.

P70

Immunohistochemical Localisation of a Calcium-Activated Chloride Channel (DOG-1) in Human Epithelial Salivary Gland Tumours: A Reappraisal

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The calcium-activated chloride channel anoctamin-1 (DOG-1) is localised in salivary glands and their tumours, but details of localisation vary, patterns are complex and functional interpretations have drawn little attention. These are pursued here. Formalin-fixed, paraffin-embedded, surgical specimens from 65 benign and malignant epithelial salivary tumours were investigated by immunohistochemistry using a monoclonal, anti-DOG-1 antibody (Clone K9, Leica Microsystems®). DOG-1 was localised to all non-oncocytic tumour types examined. Frequency, intensity of staining and relationship with cell phenotype varied between types and areas of the same tumour. Most frequent (51%) was variably strong, apical membranous staining of cells lining variably sized, rigid or collapsed, luminal structures of the tumours, being least common in basal cell adenoma (BCA). These cells showed nondescript, serous (acinic cell carcinoma - ACC) or mucous (mucoepidermoid carcinoma - MEC) phenotypes. Less frequent was staining of non-luminal cells. It was cytoplasmic, in spindled (31%) or cuboidal (22%) cells adjacent to stroma; or intercellular membranous (31%). Stained spindled cells preferentially were in the pleomorphic adenoma (PA) family, adenoid cystic carcinoma and polymorphous adenocarcinoma; stained cuboidal cells were prominent in occasional BCAs; intercellular membranous staining was appreciated in PA, ACC and MEC. Co-excision of staining patterns (34%) was variously seen in benign and, except for cystadenocarcinoma, malignant tumours (secretory carcinoma included). In adjacent glands, DOG-1 decorated acinar, intercellular canaliculi and lumina of intercalary ducts; luminal staining of striated or collecting ducts was sporadic. Expression of DOG-1 in luminal cells of epithelial salivary tumours may reflect secretory events and differentiation akin to normal glands. Expression in non-luminal cells, if intercellular, suggests inchoate lumina; it is difficult to explain, if cytoplasmic.

P71

Early Carcinoma Ex-Pleomorphic Adenoma, Diagnostic and Clinical Implications: A Case Report

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Carcinoma Ex-Pleomorphic Adenoma (CEPA) is an epithelial and/or myoepithelial malignant neoplasm that arises from a primary or recurrent pleomorphic adenoma (WHO 2017). The prognosis of an individual lesion is dependent on its degree of invasion. Widely invasive lesions are very aggressive with poor 5 year-survival outcomes. Contrastingly early lesions are associated with excellent prognosis and low rates of metastasis or recurrence. Accurate diagnosis with qualification of the degree of invasion is therefore of the utmost importance clinically. CEPA draws the attention of the literature because of the controversies surrounding the definition of the early lesions. The currently accepted view is that early CEPA encompasses everything from intraductal lesions, to CEPA with capsular invasion of less than 6mm, although this is hotly contested. Furthermore, there have been interesting recent developments in understanding the early steps in the oncogenic pathways in early CEPA. The new characterisation of two morphologically distinct histopathological subtypes of intraductal carcinoma, clinging and solid types, has given rise to the hypothesis that CEPA may develop in a manner similar to breast cancers that arise from DCIS.
Here we present a case of early CEPA of the left parotid gland in a 73 year-old man who presented with a painless mass dating months. We use this case as a platform to discuss the diagnostic challenges posed by early CEPA and to review the recent developments in the literature regarding the early steps of oncogenesis.

P72

Neuroendocrine Carcinoma of the Epiglottis: A Case Report of a Rare Entity with Assessment of Grading Parameters

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Neuroendocrine tumours (NETs) can originate in most organs of the body but are most frequently recorded in the gastrointestinal tract or lungs while being rare in the head and neck region. NETs in the larynx are almost invariably primary in origin. Although the most common non-squamous tumour of the larynx, they account for < 1% of all laryngeal neoplasms. In contrast to larynx SCC, NETs are supraglottic in location rather than the glottis because of abundant neuroendocrine cells. We discuss a case of this rare entity.
A 72 year old lady presented with hoarseness of voice and complaint of food sticking in the throat. Laryngoscopy demonstrated right vallecular irregularity. On radiology a 1.7 cm polypoid, epiglottic lesion was noted. Incisional biopsies confirmed a malignant lesion with nested architecture and tubules with a few solid areas which was positive for CD56, synaptophysin and chromogranin. A diagnosis of a high grade primary neuroendocrine carcinoma of the epiglottis was made and surgical resection advised after MDT discussion. We also take this opportunity to assess the categories and grading systems in place for Head and Neck NETs as currently they differ between organ systems and cause considerable confusion. Head and Neck NETs are rare and need further exploration for potential grading parameters and their assessment for prognostic implications. We suggest drafting of a uniform classification framework for NETs to reduce inconsistencies and contradictions due to the various systems in use.

P73

An Audit of Thyroid Cytology Reporting Standards in a University Teaching Hospital

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Purpose of study: 40% of the population have thyroid nodules, while the incidence of thyroid malignancy is 2-4%. Fine needle aspiration (FNA) helps estimate the malignancy risk of a thyroid nodule and provide guidance on if histology is required. It is recommended that all thyroid cytology reports are clearly categorised using a numerical cytology category. This allows for diagnostic classification. The Royal College of Pathologists recommend audit of reporting categories and outcomes of thyroid cytology.
Methods: Over a 6-month period, we analysed the sensitivity, specificity and diagnostic accuracy of thyroid FNA at a university teaching hospital. We studied the proportions of various Thy grades and determined which were encountered most prior to neoplastic and non-neoplastic histology.
Summary of results: 95 FNA reports were analysed. 6% did not reference a Thy grade. 11% of FNA samples were non-diagnostic. The most frequently encountered Thy grade was Thy 2 (47%). There were 25 FNA cytology cases with subsequent histology. The accuracy of thyroid FNA in differentiating neoplastic and non-neoplastic histology was as follows: 83% sensitivity, 86% specificity, 94% positive predictive value, 67% negative predictive value, 84% diagnostic accuracy. The most frequently encountered Thy grade preceding neoplastic histology was Thy 3 (67%). When Thy 3 cytology was followed by histology, 92% of cases confirmed a neoplastic process. The most frequently encountered Thy grade preceding non- neoplastic histology was Thy 2 (71%).
Conclusions: Audit of a cytology service is important to ensure adequate performance levels and highlight areas for improvement. The Royal College of Pathologists targets for sensitivity, specificity and diagnostic accuracy were met by this institution. Thyroid cytology aids in the provision of accurate diagnostic and prognostic information to clinicians, which helps improve patient care.

P74 *

Inflammatory Mediators Drive Tumorigenesis in Pituitary Adenomas of the PIT1 Lineage

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Purpose of study: Seven subgroups of pituitary adenomas (PAs) have been identified using methylation profiles (1). Four subgroups matched hormone profiles only (ACTH, LH/FSH, PRL and TSH-producing PAs), while growth-hormone-secreting tumours (GH-PAs) were further grouped into sparsely (SG) and densely granulated A and B (DG-A and DG-B). Using these data, we aimed to identify interaction hotspots for each subgroup compared with normal anterior pituitary (NP).
Methods: We used the Epigenetic Module ('EpiMod') package, which identifies genes with differential promoter methylation ('seeds'). Subnetworks ('hotspots') associated with seeds are subsequently identified if statistically significant ($p<0.05$) using a protein interaction network.
Results: Tumours of PIT1 lineage (GH-PAs, PRL-PAs and TSH-PAs) showed predominant hypo-methylation compared to normal, whereas ACTH-PAs and LH/FSH-PAs showed hypermethylation. No seeds were shared by all subgroups. However, PIT1 tumours showed multiple common seeds: 10 seeds were shared by at least two PIT1 groups. When SG, DG-A and DG-B were compared with NP, 6 seeds were common to these three groups (and all were included in the group of ten seeds described above). Gene set enrichment analysis for this group of ten seeds found pathways regulating degradation of extracellular matrix and immune response. Minimum overlap was observed between seeds identified in PIT1 tumours and LH/FSH-PAs and/or ACTH-PAs: 1 was shared between ACTH- and GH- and TSH-PAs and 2 between LH/FSH- and TSH-PAs. LH/FSH- and ACTH-PAs shared 4 seeds.
Conclusions: PIT1 tumours show striking hypomethylation. Seeds identified in PIT1 tumours are enriched for inflammatory pathways unlike LH/FSH- or ACTH-PAs, suggesting that these pathways may drive tumorigenesis in PIT1 tumours. Validation using PCR and immunohistochemistry is in progress.
References: 1. Capper, D. et al. 2018. Nature 555, 469-474
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P75

Clinico-Pathological Co-Occurrence of Fahr's Disease and Dementia with Lewy Bodies

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Purpose of the study: To describe the clinic-pathological findings of a case of Fahr's disease (FD) co-occurring with dementia with Lewy bodies (DLB), and to identify other cases describing this co-occurrence.
Methods: Retrospective review of the patient's clinical records and pathological examination of the patient's brain. Pubmed search to identify available autopsy reports of FD patients, with identification of reports describing co-existing Fahr's and Lewy body pathology.
Summary of results: The patient presented with visual hallucinations, fluctuating confusion and Parkinsonism, leading to a presumptive diagnosis of DLB. Subsequent CT scan showed extensive bilateral parenchymal calcifications, suggestive of FD. DNA sequencing identified a novel missense variant (c.92A>T p.ASN31Ile) in the SLC20A2 gene, a gene known to be associated with FD. This change has not been previously recorded in other genetic repositories, and in silico analyses classified it as disease-causing. The patient died aged 77, 4 years after symptom onset. Neuropathological examination revealed, macroscopically and microscopically, extensive calcification in the striatum, globus and cerebellar white matter. There was also neuronal loss in the substantia nigra and residual neurones contained alpha-synuclein-positive Lewy bodies. The neuropathology was therefore consistent with DLB and FD, in line with the clinico-radiological features. A literature review identified 3 other cases of co-existing Fahr's and Lewy body pathology, thus the frequency of dual pathology (44%) is higher than expected by random association.
Conclusions: We present the clinico-pathological findings of a case of combined FD and DLB, associated with a novel pathogenic mutation. Further studies are needed to determine whether alpha-synucleopathy is linked mechanistically to FD and/or represents a phenotypic subtype. Identifying factors driving this co-existence could reveal insights into the pathogenesis of both diseases.

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Variations of Hippocampal Spherical Tau Inclusion in Different Tauopathies

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Background: Tauopathies are defined by the pathological aggregation of hyperphosphorylated tau proteins in various brain regions. Tau pathology can be categorised as three microtubule-binding repeats (3R) or four repeat (4R) predominant, or mixed. Recent studies suggested that 4R globular tau found in the dentate gyrus could be a new tauopathy variant.
Aims: To investigate the composition of globular tau found in the dentate gyrus in patients with Alzheimer's disease (AD), Progressive supranuclear palsy (PSP) and Corticobasal degeneration (CBD).
Method: AT8 and p62 Immunostained hippocampus slides were retrieved and assessed. Both semi-quantitative and quantitative scoring systems were used to rate the frequency of globular tau in the dentate gyrus. Hippocampal slides with a globule score higher than 'frequent' were immunostained with 3R and 4R antibodies.
Results: In AD cohort, the results showed a strong correlation between disease duration and globule frequency: the longer the disease duration, the higher the globule number. An opposing trend appeared in PSP cohort. Moreover, high tau density in the dentate gyrus increased the likelihood of globule presence in both AD and PSP cohort. 3R and 4R immunostainings showed that AD tau globules were 3R predominate and Pick-body like, whilst PSP tau globules were 4R predominate.
Conclusion: These results show the composition of globular tau in the dentate gyrus varies between different tauopathies. In addition to that, disease duration has a significant role in influencing globule frequency.
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