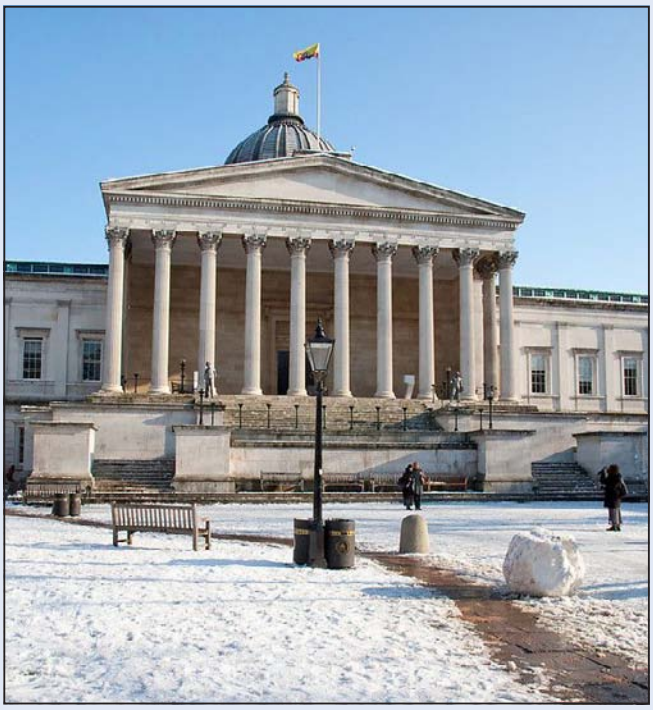

Abstracts

WINTER MEETING

Epigenetics – Understanding Disease and Guiding Therapies

25 – 26 January 2018

Joint Meeting with the Royal Society of Medicine
209th Scientific Meeting of the Pathological Society of Great Britain & Ireland

Hosted by
The Research and Clinical Departments of Pathology
University College London and
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PHOTOGRAPHS

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Invited Speaker Abstracts

S1

Bioinformatics for Personalized Medicine: Looking Beyond the Genome

Ⓟ C Bock

CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

The complexity of the human body requires trillions of individual cells to integrate, interact, and strike the right balance between stability and plasticity. Key mechanisms underlying this extraordinary feat of self-organization are encoded in the human genome, yet there are additional levels of regulation that are operate on top of the genomic DNA sequence, collectively referred to as the epigenome.
International consortia have mapped the human genome, epigenome, and transcriptome in hundreds of cells types. These maps are now being refined by ongoing single-cell sequencing projects, which will eventually give rise to a comprehensive catalog of all cells in the human body. Contributing to these consortia and building on their data, we investigate the relevance of the human epigenome for personalized medicine, focusing on better diagnostics, adaptive therapies, and disease modeling.
We have developed bioinformatic methods for analyzing and interpreting DNA methylation data (reviewed in: Bock 2012 Nature Reviews Genetics), which contribute to their use as epigenetic biomarkers. Epigenome analysis will have an important role to play for a forward-looking and prediction-based approach to cancer therapy, which is inspired by the impact of computational methods on HIV therapy (reviewed in: Bock & Lengauer 2012 Nature Reviews Cancer).
In our ongoing work, we co-develop computational and experimental methods for epigenome, transcriptome, and multi-omics profiling in single cells (reviewed in: Bock et al. 2016 Trends in Biotechnology). We have also established an assay and bioinformatic methods for CRISPR single-cell sequencing, which enables the large-scale functional analysis of regulatory mechanisms (Datlinger et al. 2017 Nature Methods), and we are applying this technology to dissect the role of the human epigenome for cancer and immune diseases.
Funding: CB is supported by a New Frontiers Group award of the Austrian Academy of Sciences and by an ERC Starting Grant.

S2

The Genetics and Pathology of Melanoma: From Mouse to Human and Back Again

Ⓟ DJ Adams

Wellcome Trust Sanger Institute, Hinxton, UK

Great progress has been made in understanding the somatic genetics of melanoma and in explaining why some people are more likely to develop the disease. Melanoma also represents a bench-to-clinic translational success story with checkpoint inhibitors now becoming standard of care. I will discuss the latest technology for analysing cancer genomes and the use of gene-editing to study melanoma in human cells and mice. Although the focus will be melanoma the approaches discussed are applicable across tumour types.

S3

The Lung TRACERx Study

Ⓟ M Jamal-Hanjani[1]; C Swanton[2]; and the TRACERx Consortium

[1]UCL Cancer Institute, London, UK; [2]UCL Cancer Institute and Francis Crick Institute, London, UK

The lung TRACERx study aims to track the evolution of non-small cell lung cancer from diagnosis to relapse and to determine the relationship between intratumour heterogeneity and clinical outcome. Analysis of the first 100 early stage tumours has demonstrated a significant correlation between intratumour heterogeneity, mediated by chromosomal instability, and increased risk of recurrence or death. Patients who develop metastatic disease in TRACERx are recruited into the PEACE post-mortem study facilitating the paired analysis of primary and metastatic tumour with the aim of identifying patterns of metastatic disease, potential mechanisms of drug resistance and exploring in greater detail the process of tumour evolution.

S4

The Human Cell Atlas

Ⓟ MJT Stubbington

Wellcome Trust Sanger Institute, Cambridge, UK

The Human Cell Atlas is a new international initiative that aims to define all human cell types in terms of their gene expression and other distinctive molecular characteristics. This information will be linked with features such as location and morphology to provide a comprehensive reference map of the molecular states of cells within healthy human tissues.
This will provide an extremely valuable resource to empower the global research community to systematically study the biological changes associated with different diseases, identify where genes associated with disease are active in our bodies, analyse the molecular mechanisms that govern the production and activity of different cell types, and understand how different cell types combine and work together to form tissues.
To achieve this, disparate fields of expertise in biology, medicine, genomics, technology development, and computation (including data analysis, software engineering, and visualization) will need to come together in a coherent, concerted way. Furthermore, an international effort must be able to compare across diverse cell types and tissue types in consistent ways, while studying samples from diverse human communities.
I will introduce this nascent initiative and discuss how it will be achieved by groups working together throughout the world.

S5

Genomic Approaches to Pathogen Detection in Histopathological Material

Ⓟ J Breuer

UCL, London, UK

Classically, the identification of infectious agents in disease has relies on both catch-all and targeted methods. Catch-all methods include culture, electron microscopy and histology for detection of pathognomonic features associated with some infections. These methods although unbiased, are relatively insensitive for pathogen detection. Targeted methods which identify known pathogens that are potentially associated with the disease include detection of pathogen nucleic acid by PCR, in situ hybridisation and immunohistochemistry for pathogen proteins. Targeted methods can be highly sensitive but are by definition biased by prior knowledge. To overcome these limitations we have applied metagenomic sequencing technologies to pathogen detection and discovery in brain biopsies obtained from cases of encephalitis for which conventional methods have not yielded a diagnosis. Positive findings have been confirmed by immunohistochemistry. Here I present our experience in optimising the methods for use in both fresh and formalin fixed material, the results we have obtained and discuss the place of these approaches in modern-day diagnostics.

S6

Twins, Microbiomes and Personalised Health

Ⓟ TD Spector

King's College London, London, UK

The microbiome is the community of 100 trillion microbes that live in our colon that are like a virtual organ. This organ is key to our digestion, appetite, mood, metabolism, and control of our immune system. It is also key to how we respond to immunotherapy and chemotherapy. The TwinsUK cohort of 12,000+ twins has been running for nearly 25 years and is now the most intensively studied group of humans on the planet (www.twinsuk.ac.uk). Having deep sequence, metabolites, epigenetics, immune traits and dietary and health data, in 2012 we added stool collection for 16S microbiome, metagenomes and metabolomics. We are currently using the microbiome data and cohort to provide novel measures of health, such as the level of microbial diversity and a new measure — the microbial health index and how this impacts overall health outcomes. Our twin work has also enabled us to gain insights into the microbiome and immune interactions of the upper colon and small intestine via colonoscopy and interventions. Every medical professional needs to know about maintaining a healthy microbiome from birth to death.

Plenary Oral Abstracts

PL1

The Molecular Pathology of Infant Gliomas

Ⓟ MT Clarke[1]; D Jones[2]; DM Carvalho[1]; A Mackay[1]; E Izquierdo[1]; D Hargrave[3]; AS Moore[4]; S Popov[5]; TS Jacques[6]; C Jones[1]

[1]Institute of Cancer Research, London, UK; [2]DKFZ German Cancer Research Center, Heidelberg, Germany; [3]Great Ormond Street Hospital, London, UK; [4]The University of Queensland, Brisbane, Australia; [5]University Hospital of Wales, Cardiff, UK; [6]UCL Great Ormond Street Institute of Child Health, London, UK

Infant gliomas have largely been considered as simply early-onset examples of the same tumour types found in older children. However, existing survival data shows that high grade gliomas (HGGs) in infants have a better overall survival compared to their older counterparts, whereas in low grade gliomas (LGGs) they do worse. This indicates that infants may represent a tumour subgroup where the grading does not reflect the biology of the tumour. We have to-date collected 72 cases of diffuse glioma (WHO grades II-IV) occuring in children aged <4 years and performed histological review, Illumina EPIC methylation profiling, and a custom gene fusion sequencing panel. After exclusion of misclassified *BRAF:KIAA1549* fusion positive cases (n=5), we observed using the latest version of the Heidelberg brain tumour classifier that the majority of cases do not fall into recognised tumour subgroups by methylation profiling. A large proportion fell into a loose subgroup currently termed "desmoplastic infantile ganglioglioma 2" (DIG2, n=19), or were unclassifiable (n=13). The former group are found most frequently in infants aged ≤1 year and are associated with *NTRK1/2/3* and *ALK* fusions identified by fusion panel sequencing. When well-characterised brain tumour entities and subgroups are excluded from the cohort, consensus clustering suggested three novel infant subgroups. Histological review shows that 16/42 cases contained areas with a spindled cell morphology. We further established a novel patient-derived DIG2 cell culture with *ETV6:NTRK3* fusion, which showed exquisite *in vitro* sensitivity to a panel of Trk inhibitors including Larotrectinib (LOXO-101), making such patients excellent candidates for upcoming clinical trials of this compound. In conclusion, the clinical, histological and molecular features of infant gliomas suggests the presence of novel tumour subgroups which may have targetable driving alterations. Additional work is underway to further characterise these subgroups.

PL2

What Makes an Expert Pathologist? — Histopathologist Features Predictive of Diagnostic Concordance at Expert Level Amongst a Large International Sample of Pathologists Diagnosing Barrett's Dysplasia

Ⓟ M Jansen[1]; MJ Van der Wel[2]; HG Coleman[3]; SL Meijer[2]

[1]University College London Hospital, London, UK; [2]Academic Medical Centre, Amsterdam, Netherlands; [3]Queen's University Belfast, Belfast, UK

Purpose: Histopathological diagnosis of dysplasia in Barrett's oesophagus is the gold standard for patient risk stratification, but is subject to significant interobserver variation. We investigated histopathologist features that predict diagnostic concordance at expert level amongst a large international cohort of gastro-intestinal (GI) pathologists.
Methods: An online scoring environment was developed for participants (n=55 GI-pathologists) from over 20 countries to grade a case set of 55 digitised BO biopsies encompassing the complete spectrum from non-dysplastic Barrett's oesophagus (NDBO) to high-grade dysplasia (HGD). Detailed histopathologist demographic data (experience, centre volume, etc.) was obtained through an online questionnaire. We also quantified the impact of p53 immunohistochemistry (IHC) on diagnostic concordance. Finally, low-pass whole genome sequencing (WGS) was carried out to correlate molecular complexity to diagnostic concordance.
Summary: We recorded over 6,000 case diagnoses. We found excellent concordance for NDBO (643 of 816 diagnoses; 79%) and HGD (544 of 765 diagnoses; 71%) and intermediate concordance for LGD (382 of 918; 42%) and IND (70 of 306; 23%). Significant misdiagnoses (i.e. NDBO overstaged as HGD, or HGD understaged as NDBO) were rare (9 of 816 diagnoses; 1.1%; and 17 of 765 diagnoses; 0.6%). Addition of p53 IHC significantly increased diagnostic concordance. Regression analyses revealed histopathologist predictors of diagnostic concordance at expert level and allowed us to model optimal revision strategies based on case and pathologists characteristics.
Conclusions: Our study is the largest carried out thus far to quantify pathologist dependent factors predictive of diagnostic concordance using digital pathology. These data will allow rational formulation of quality assurance criteria for guideline development. Our study method is highly scalable and broadly applicable to any area of histopathologic diagnostic uncertainty.

PL3

Bone Marrow Mesenchymal Cells Deficient in TNF Receptor-Associated Factor 3 Attract RANKL-Expressing CD19+B220++IgM+ Recirculating B Cells via CXCL12/CXCR4 Signaling to Contribute to Age-Related Bone Loss

Ⓟ BF Boyce; J Li; L Xing; Z Yao

University of Rochester Medical Center, Rochester, New York, United States

Aging is associated with increased RANKL/NF-kB-induced bone loss, but the major cellular sources of RANKL in bone marrow (BM) and bone during aging are unknown. We flow-sorted BM and bone-derived cells (BDCs) from 3-m-old C57BL/6 mice. The number of RANKL+B cells is significantly higher than RANKL+T cells and mesenchymal stromal cells (MSCs) in BM and osteoblasts (OBs) in BDCs. ~86% RANKL+B cells are B220(++) and ~90% are IgM+, with ~80% RANKL+B cells enriched in the CD19+B220++IgM+ (recirculating) subpopulation, which was higher in BM from 18-m than 3-m-old mice (49% v. 25%). TNF receptor-associated factor 3 (TRAF3) negatively regulates NF-kB signaling and could limit RANKL-induced resorption during aging. We found that TRAF3 protein levels in OB and osteoclast (OC) lineage cells decrease in mouse bone with age. We generated mice with TRAF3 deleted in OCs (using Lys-MCre) and OB/MSCs (Prx-1Cre). Both conditional knockout (cKO) mice developed early onset osteoporosis with increased bone resorption. Prx-1 cKO mice had increased RANKL expression in BM cells and significantly more BM recirculating cells; these can be recruited to BM by CXCL12/CXCR4 signaling. Cxcl12 mRNA levels in MSCs were higher (7-fold) and CXCL12 protein levels were 16-fold higher in BM cells from aged mice, which also had 2-fold more CXCL12+ MSCs. Prx1-cKO mice had ~30-fold higher Cxcl12 mRNA levels in MSCs and significantly more CXCL12+ cells in tibial sections than controls. We found significantly more CXCR4+recirculating B cells in BM from aged than young mice and from Prx-1 cKO than control mice. These data suggest that CD19+recirculating B cells are the predominant RANKL-expressing population in BM promoting OC formation during aging, as TRAF3 levels fall, and that TRAF3 deletion in OB/MSCs causes RANKL+B cell accumulation in BM via enhanced CXCL12/CXCR4 signaling. Inhibition of TRAF3 degradation in MSCs and of CXCR4+recirculating B cell recruitment to BM could reduce age-related osteoporosis.

PL4

Clonal Evolution and the Development of Colon Adenomas and Carcinomas

Ⓟ WCH Cross[1]; TA Graham[1]; I Tomlinson[2]; S Leedham[3]; NA Wright[1]

[1]Barts Cancer Insititute, QMUL, London, UK; [2]Institute of Cancer and Genomic Sciences, Birmingham, UK; [3]Wellcome Trust Centre for Human Genetics, Oxford, UK

Background: The transformation of pre-malignancy to cancer remains incompletely understood, but involves positive selection of driver mutations — exemplified by the Vogelstein model. By measuring heterogeneity these selection forces can be exposed, revealing previously unseen details of how cancers develop. We present an in-depth analysis of the heterogeneity of colon tumours and suggest that selection forces act differentially upon adenomas and carcinomas. We also provide evidence for stabilizing selection forces, which appear to constrict the karyotypes that are viable within carcinomas.
Methods: We performed bioinformatic and phylogenetic analysis of 27 carcinomas and adenomas. A specialist bioinformatics pipeline was used to jointly call single nucleotide variants and copy number aberrations. We assessed positive selection through the clonal distribution of known driver mutations and by phylogenetics.
Results: We found a high degree of nucleotide heterogeneity in all tumours, and surprisingly, that diversity was higher in adenomas. Regional driver mutations were present in 30% of adenomas, but no carcinomas. Genome doubling, high levels of aneuploidy and p53 loss, were found regionally in a small number of adenomas. Further analysis demonstrated that in carcinomas the diversity of aneuploidy is strikingly homogeneous, meaning there is a 'core' chromosome configuration defining each lesion.
Conclusions: We suggest that positive selection is variegated in adenomas, meaning that clones can co-exist within a cell population. By contrast, carcinomas appear to be homogenous. A genetic bottleneck during the adenomatous phase, would explain these data since competitive clones in the adenoma could become extinct, reducing diversity. We suspect that the homogeneity of cancer karyotypes is not only a reflection of a genetic bottleneck, but also the result of stabilizing selection, which dramatically reduces deviation in chromosomal copy changes.

Poster Abstracts

P1

Myoepithelial Cell-Associated Galectin-7: Functional and Clinical Relevance in DCIS Progression

Ⓟ N Allen; M Allen; J Gomm; JL Jones

Barts Cancer Institute, London, UK

Background: A breast screening review highlighted the need to reduce overdiagnosis. DCIS contributes significantly to this. As DCIS evolves there are concomitant changes in the ductal microenvironment. Our group previously identified changes in non-neoplastic myoepithelial cells that convert these tumour-suppressors to tumour promotors. The study aims to investigate the functional and clinical significance of myoepithelial Galectin-7, a protein shown to have anti-apoptotic function, and to use this to develop a biomarker panel to risk stratify DCIS.
Methods: Galectin-7 expression was assessed by IHC in 2 groups, each with 23 cases: pure DCIS(low risk model) and DCIS with associated invasion(high risk model). Individual DCIS ducts were scored as Galectin-7 positive, heterogeneous or negative. Normal primary myoepithelial cells isolated from reduction mammoplasty were used as a model to investigate Galectin-7 function. These cells endogenously express high levels of Galectin-7. Galectin-7 was silenced using siRNA and apoptosis assessed using cleaved PARP and caspase-3. Phosphoproteomics and RNA-seq have been undertaken to analyse the global impact of Galectin-7.
Results: 1926 DCIS ducts were scored for IHC expression of Galectin-7. Pure DCIS and DCIS with invasion had 338 and 144 positive DCIS ducts, respectively(p=0.0014). Pure DCIS and DCIS with invasion had 99 and 646 negative DCIS ducts respectively(p=0.0002). Remaining ducts were heterogeneous. Significant knockdown of Galectin-7 was achieved in primary myoepithelial cells. Western blotting demonsrated increased expression of cleaved PARP and caspase-3 in Galectin-7 knockdown cells compared to non-target controls, indicating reduced Galectin-7 increases apoptosis.
Conclusion: Galectin-7 loss in DCIS, is associated with a more aggressive DCIS phenotype. Galectin-7 shows translational promise in the development of an IHC panel for DCIS risk stratification. Galectin-7 is currently being validated using the UK DCIS trial

P2

Lysosomal Protective Protein/Cathepsin A (CTSA) is an Independent Prognostic Factor in Breast Ductal Carcinoma *in situ* (DCIS)

Ⓟ MS Toss; I Miligy; A Alkawaz; CC Nolan; M Diez-Rodriguez; IO Ellis; AR Green; EA Rakha

Academic Pathology, Division of Cancer and Stem Cells, School of Medicine, The University of Nottingham, Nottingham, UK

Background and Aim of the Study: Cathepsin A (CTSA) is a key regulatory enzyme for galactoside metabolism. Additionally, it has a distinct proteolytic activity and plays a role in tumour progression. CTSA is differentially expressed at mRNA level between breast ductal carcinoma in situ (DCIS) and invasive breast carcinoma (IBC). Here, we aimed to characterise CTSA protein expression in DCIS and evaluate its prognostic significance.
Methods: Tissue microarray (TMA) was constructed from a large cohort of DCIS that have available paraffin blocks with representative tumour tissue (n=750 for pure DCIS and n=239 for DCIS associated with IBC (DCIS/IBC)). TMA sections were stained for CTSA immunohistochemically and scored using H-score following robust validation of staining specificity.
Results: High CTSA expression was observed in 11% of pure DCIS. High expression was associated with features of poor prognosis including younger age, symptomatic presentation, higher nuclear grade, comedo necrosis and hormone receptor negativity. High CTSA expression was associated with shorter recurrence free interval (RFI) (p=0.0001). In multivariate survival analysis for patients treated with breast conserving surgery; CTSA was an independent predictor of shorter RFI (p=0.005). DCIS associated with IBC showed higher CTSA expression than pure DCIS (p=0.0001). In DCIS/IBC cohort; CTSA expression was higher in invasive component than DCIS component (p=1.0x10-13).
Conclusion: CTSA is not only associated with aggressive behaviour and poor outcome in DCIS but also a potential marker to predict co-existing invasion in DCIS.

P3

Targeting Protein for Xenopus Kinesin-Like Protein 2 (TPX2) Expression is Associated with Poor Outcome in Breast Ductal Carcinoma *in situ* (DCIS)

Ⓟ I Miligy[1]; A Gaber[2]; MS Toss[1]; AA Al-Kawaz[1]; CC Nolan[1]; MA Diez Rodriguez[1]; IO Ellis[1]; AR Green[1]; EA Rakha[1]

[1]Nottingham City Hospital, University of Nottingham, Nottingham, UK; [2]Faculty of Medicine, Menoufia, Egypt

Background: The mechanisms underlying tumorigenesis and progression of breast ductal carcinoma in situ (DCIS) is still not clear. Identification of progression markers is crucial for determining which lesions are likely to become invasive. Therefore, it is a key point in both discovering new therapeutic targets and improving prognosis of DCIS patients to search for new transferred molecular markers. Targeting protein for Xenopus kinesin-like protein 2 (TPX2) is a prohibitin located on 20q11.21, strictly regulated by cell cycle, with involvement in microtubule-associated proteins formed by spindle apparatus in mitosis. TPX2 is differentially expressed at the mRNA level between breast DCIS and invasive breast carcinoma (IBC) and contributes to promote the proliferation of breast cancer cells. We aimed to investigate TPX2 role in DCIS progression.
Patients and methods: 1,057 consecutive DCIS patients treated in Nottingham between 1990 and 2012 were prepared as tissue microarrays. Patients' clinical information, management and follow-up data were retrospectively collected. The expression of TPX2 was assessed immunohistochemically and assessed with clinicopathological parameters.
Results: In pure DCIS tumours, high nuclear TPX2 expression was associated with high tumour grade (p=0.02), negative Her2 status ($p=2x10^{-5}$) and shorter recurrence free interval (RFI) ($p=2.1x10^{-6}$). Multivariate analyses indicate that independent predictors of DCIS recurrence are high TPX2 expression ($p=1x10^{-5}$, HR=3.6 and 95%CI: 2.0-6.4), larger DCIS size and high nuclear grade. DCIS associated with IBC showed higher TPX2 expression than pure DCIS ($p=5x10^{-10}$). In DCIS/IBC cohort; TPX2 expression was higher in DCIS component than in invasive component ($p=9x10^{-11}$).
Conclusion: TPX2 is not only associated with aggressive tumour type and poor outcome in DCIS through its proliferative activity but also a potential marker to predict co-existing invasion in DCIS.

P4

Characterisation of HER2 Status in DCIS Using Immunohistochemistry (IHC) and Chromogenic *in situ* Hybridisation (CISH)

Ⓟ I Miligy[1]; A Gaber[2]; MS Toss[1]; AA Al-Kawaz[1]; CC Nolan[1]; MA Diez-Rodriguez[1]; H Burrell[3]; IO Ellis[1]; AR Green[1]; EA Rakha[1]

[1]Nottingham City Hospital, University of Nottingham, Nottingham, UK; [2]Faculty of Medicine, Menoufia, Egypt; [3]Nottingham Breast Institute, Nottingham University Hospitals, Nottingham, UK

Background: Previous studies have reported high percent (up to 60%) of ductal carcinoma in situ (DCIS), the non-obligate precursor of invasive breast cancer (IBC), being HER2+. However, the frequency of HER2+ in IBC is much lower and ranges from 10% to 20%. The aim of this study is to characterise HER2 status in DCIS and assess its prognostic value.
Methods: Tissue microarrays (TMAs) were constructed from a large and annotated series of DCIS comprising pure (n=777) and mixed (DCIS associated with IBC; n=239) DCIS. HER2 status was evaluated at the protein level using immunohistochemistry (IHC) and the gene levels using chromogenic in situ hybridisation (CISH) according to the published HER2 guidelines recommendation for IBC.
Results: HER2 negative (0/1+) DCIS tumours represented 76.3% of the whole cohort, whereas HER2 2+ (equivocal) was present in 15.4% and HER2 3+ status was identified in 8.3%. CISH did not detect HER2 gene amplification in 79.7% of DCIS cases. In IHC equivocal cases, HER2 amplification (defined as tumours showing a mean HER2 gene copy number of more than or equal to 6 signals per nucleus) was confirmed by CISH in 74%. CISH confirmed high copy number of HER2 gene in all IHC 3+ cases. The final HER2+ status of pure DCIS, confirmed by CISH, represented 20.4% of the total cohort. In mixed DCIS cases, HER2 amplification of the DCIS component was detected in 14.9% with amplification of invasive component represented only 12.6%. HER2+ DCIS was associated with high nuclear grade ($p= 6.3x10^{-13}$), comedo type DCIS (p= 0.005), larger tumour size ($p= 2x10^{-6}$) and negative hormone receptor status ($p= 3.4x10^{-21}$).
Conclusions: Our results indicate the frequency of HER2 positivity in DCIS is comparable to IBC and that HER2+ DCIS is associated with features of poor prognosis. Similar to IBC, the majority of HER2 overexpression in DCIS is driven by gene amplification.

P5

Spindle Cell Adenomyoepithelioma of the Breast: A Case Report

Ⓟ R Khiroya; K Sherring; E Borg; M Falzon; C Wells

University College London Hospital, London, UK

We describe a case of spindle cell adenomyoepithelioma of the breast, which was diagnosed on needle core biopsy. A 79 year old female with a history of transverse myelitis was referred for breast surgical opinion to rule out breast malignancy as a cause for her transverse myelitis. Mammograms revealed a 7mm ill-defined dense mass in the right upper outer breast region with indeterminate appearances. The lesion was biopsied under ultrasound guidance.

Core biopsies showed a lesion composed predominantly of small fascicles of cytologically bland spindle cells with intervening collagenous stroma. Scattered tubules were also present. There was no evidence of cytological atypia, mitotic activity or necrosis. Immunohistochemistry showed the spindle cell component was positive for smooth muscle myosin, CK5 and S100, consistent with myoepithelial origin. The cells lining the tubules were positive for CK19, consistent with luminal origin. A diagnosis of spindle cell adenomyoepithelioma was made. The patient subsequently underwent a vacuum excision to remove the lesion. Histology showed similar features to those seen in the original biopsy. The patient has been well since the excision with no signs of recurrence.

Adenomyoepithelioma is a rare tumour of the breast. The spindle cell adenomyoepithelioma is a variant in which the myoepithelial component predominates, and is rarer still, with less than 10 cases having been described in the literature. Most cases are benign, and complete excision is curative. Spindle cell lesions of the breast are a challenging area, and this case raises awareness of an uncommon tumour.

P6

Investigation of Capacity of Exogenous dsRNA to Cause Chemoimmunogenic Killing of p53 Mutant Breast Cancer Cells

Ⓟ H Navabi[1]; L Campbell[2]; B Jasani[3]

[1]Velindre Hospital, Cardiff, UK; [2]Cancer Research Wales, Cardiff, UK; [3]Targos Molecular Pathology GmbH, Kassel, Germany

Background: Chemoimmunogenic cancer cell death is associated with enhanced anti-cancer immunity (Cancer Cell 2015; 28:690). It operates through genotoxic stress induced endogenous dsRNA driven autocrine/paracrine type I interferon (INFI) pathway activation triggering killing of cancer cells and innate/adaptive immune responses (Nat Med. 2014; 20:1301; Oncotarget 2015; 6:41600). The INFI pathway cytotoxicity appears to be selective for cells with impaired p53 function (J Virol 2005; 79: 11105).

Aim: To investigate capacity of a synthetic dsRNA analogue Ampligen to cause chemoImmunogenic cell death in breast cancer cells.

Methods: A panel of constitutive and transfected breast cancer lines with wt and impaired p53 status were treated with or without Ampligen or 5-FU (non-immunogenic). Cell viability was quantified using CellTiter-Glo® Luminescent Cell Viability Assay and induction of innate immune response probed using Western blot and Quantitative RT-PCR.

Results: Ampligen caused transient increase of TLR3, RIG-I and IFNb mRNA and protein expression in all cell lines. This response was p53 independent but unlike 5FU, Ampligen caused a highly selective, marked decrease (50%) in cell viability in p53-blocked MCF-7 and constitutively p53-null MDA-MB453 breast cancer cells. Bx795, specific inhibitor of TBK1/IKKε and intracellular type I IFN signaling pathway, abrogated the Ampligen cytotoxicity effect without alteration in TLR3, RIG-I and IFNb expression or cell proliferation rate.

Conclusion: Overall data demonstrate exogenous dsRNA capacity to cause chemoimmunogenic cell death through activation of the innate cellular Type I inteferon signaling pathway and which is paradoxically dependent upon impaired p53 status. The findings offer a novel adjuvant treatment approach for triple negative breast cancers frequently associated with dysfunctional p53 status.

P7

B3 Diagnosis in Core Breast Biopsies – Evaluation of the Outcome and Positive Predictive Value Relative to the Royal College of Pathologists Dataset

Ⓟ Y Al-Janabi; S Lower; P Davis

Mid Essex Hospital Services NHS Trust, Chelmsford, UK

Purpose of Study: This quality improvement project was undertaken to evaluate the breast core biopsies graded as B3, lesions of uncertain malignant potential, at one NHS trust over a period of one year. The results were compared with the Royal College of Pathologists dataset recommendations regarding the suggested rate for B3 lesions and their positive predictive value, with an overall aim to explore potential points for improvement in the diagnosis of B3 lesions.

Methods: Data from all core breast biopsy reports at the trust from August 2015 to August 2016 were used to identify the total number graded as B3. All B3 cases were followed up to evaluate, after excision biopsy or vacuum-assisted biopsy, how many lesions were upgraded to malignant and how many were downgraded, in order to find the positive predictive value for B3 lesions.

Summary of Results: In total, 34 cases were identified as B3, making up 4% of all core breast biopsy cases, compared to the Royal College of Pathologists recommendation of 4.5 - 8.5%. On subsequent investigation, 10 of these cases were upgraded and were found to be cancerous, giving a positive predictive value of 32%, compared to the current national median of 15%.

Conclusions: Compared to the guidelines from the Royal College of Pathologists, the proportion of lesions diagnosed as B3 in the trust was slightly under the recommended threshold, and the positive predictive value of B3 lesions in the trust was more than double the national median. These results suggest there should be a lower threshold for diagnosing B3 breast lesions to increase the likelihood of identifying those B3 lesions that are subsequently found to be cancerous. Potential points to implement are suggested to help achieve this including education, multidisciplinary team cases review and team discussions on B3 diagnoses.

P8

Grade of Breast Carcinoma in Symptomatic Patients – What are the Golden Standards to Audit Against

Ⓟ H Hashim[1]; F Alchami[2]

[1]Cardiff University, Cardiff, UK; [2]University Hospital of Wales, Cardiff, UK

Introduction: Histological grading provides a powerful prognostic and therapeutic factor in breast cancer. Nottingham Grading System is the most commonly used grading system in breast cancer assessment. The current benchmark ratio for symptomatic invasive breast cancer proposed by Royal College of Pathologist (RCPATH) is 2 : 3 : 5, (grade 1:grade 2:grade 3) referencing a paper by Elston and Ellis studying a population studied in the period between 1973-1989 in England and West Midlands.

Materials and Methods: Google Scholars search was conducted for publications between 1991 to 2016. The following keywords were included during the search: Invasive breast cancer, screen-detected breast cancer, symptomatic breast cancer, histological grade and histological grade ratio. Internal audit of grades in 2200 breast cancer in the University Hospital of Wales UHW was conducted.

Results: Audit results of UHW showed the ratio of histological grade of screen-detected subgroup is similar to NHS Breast Screening Programme yearly audit Ratio of 2 : 5: 3. The symptomatic group displays a ratio of 1 : 5 : 4. Both screen-detected and symptomatic subgroup shows a combination ratio of 1.6 : 5.1 : 3.3. The literature search of reported breast cancer grade ratio showed only two papers separating symptomatic from screening patients. The reported ratios varied between 0.6:3.4:6.1 and 1.8:5.1:3.1, and varied widely against country of origin and years studied.

Discussion: There is wide variation in the reported ratio of breast cancer grade in the UK. Whilst auditing the grade of symptomatic breast cancer is a requirement by the Royal College of Pathologists, such audit results are not available or even discussed at national level in contrary to the screening detected breast cancer. A cross centre study is required in order to establish a more up to date ratio of breast cancer grade; which will provide a gold standard for future audits.

P9

The Effect of Cancer-Associated Fibroblast Secretomes on Breast Cancer Cell Migration and Invasion

Ⓟ A Amphlett; I Goulding; L Jones

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Both intrinsic changes of breast epithelium and extrinsic changes of the immediate cellular environment are required for breast tumour progression. Cancer-associated fibroblasts (CAFs) are the most numerous cellular component of the tumour microenvironment. Evidence suggests that CAF secretomes play a key role in breast cancer progression. However, few studies have compared the effect of tumour-derived and surround-derived (>5 cm from tumour) fibroblast secretomes on breast cancer cell migration and invasion, and no studies have determined whether CAFs promote breast cancer in a subtype-specific manner.

This study used migration and invasion assays to evaluate the effect of media conditioned by tumour-derived and surround-derived fibroblasts from ER+ HER2- and ER- HER2+ tumours on breast cancer cells lines with complimentary (MCF-7: ER+ HER2-) and opposing (SKBR3: ER- HER2+) receptor statuses.

Tumour-derived fibroblast secretomes predominantly promoted migration and exclusively promoted invasion, relative to control conditions. However, differences between migration induced by tumour-derived and surround-derived secretomes was not always significant at the 5% level. MCF-7 migration was enhanced an additional 3% by fibroblast secretomes derived from tumours which shared MCF-7 receptor status. However, no difference in SKBR3 migration or MCF-7 invasion was observed dependant on receptor status.

This suggests that tumour-derived and surround-derived secretomes differ in their effect on breast cancer cell migration and invasion, and that migration of MCF-7 cells is promoted in a subtype-specific manner. Further studies are required to characterise secretomes, increase statistical power and move generalisability of results beyond these tumours and cell lines.

P10

Use of CRISPR Cas9 to Downregulate ITCH1 and Sensitise Cancer Cells to Chemotherapy and Radiation

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Purpose of Study: The e3 ubiquitin ligase ITCH has been shown to not only be an important regulator of the immune system in both mouse knockout models and humans lacking the ITCH gene, but also a regulator of many proteins associated with cancer growth and progression such as p73, LATS-1, Notch and downstream components of the TGF-beta signalling cascade. A previous study in xenografts reported that knockdown of ITCH by SiRNA increased sensitivity to doxorubicin.

Methods: In this study we utilise CRISPR-Cas9 technology to generate pancreatic cell lines with an irreversible ITCH knockdown to test the hypothesis of ITCH being a potential therapeutic target in difficult-to-treat cancer-types. Three separate lines were generated using different guide RNA sequences and ITCH knockdown was confirmed by mRNA and protein expression. One cell line was selected fro further study (dubbed IKO1a). Cells were selected for antibiotic resistance and kill curves obtained at different concentration of drug or irradiation.

Summary of Results: We compared cell viability post-treatment with gamma radiation, doxorubicin and gemcitabine in our knockdown cell line and parental MiaPaCa-2 cells. There is a significant increase in sensitivity to lower doses of radiation (below 3 Gray) and doxorubicin in the IKO1a cell line compared to the wild-type, with a decrease of approximately 30% in IC50. However, there is no significant difference between the two cell lines when treated with gemcitabine or high-dose radiation/doxorubicin suggesting that even if ITCH knockdown does sensitise cells there may be other mechanisms of death engaged at higher ;levels of injury.

Conclusions: Though the mechanism by which this phenomenon occurs is unclear, the study does lend support to the idea of targeting ITCH to increase sensitivity to existing cancer therapeutics.

P11

Electron Microscopy Shows that 5 Fluoruracil Causes Endoplasmic Reticulum Stress that may be Partly Responsible for Determining Cancer Cell Death

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Purpose of the Study: 5-Fluorouracil (5FU) usually in combination with other drugs, has been the first-line treatment in cancer, including colorectal cancer (CRC), for more than 50 years. However the majority of patients have an innate or acquired resistance. 5FU inhibition of Thymidylate Synthase (TS) causes misincorporation of metabolites into DNA leading to DNA damage and cell death. Interestingly, TS increases following treatment, leading to the question of whether ER stress could provide an alternative route to cell death. This study addressed the hypothesis that ER stress caused by 5FU may be a determinant of cell death.

Methods: Transmission EM and cell culture were performed on cell pellets using two CRC cell lines.

Summary of Results: Out of SW480 and HCT116, the former was more resistant to 5FU. HCT116s were more sensitive to Tunicamycin and Thapsigargin treatment, known ER stress inducers, than SW480s. Stereological principles were applied to EM micrographs to quantify ultrastructural morphological changes characteristic of ER stress. Untreated SW480s were found to have a higher density of mitochondria, ER, and greater degree of contact points between mitochondria and ER than their HCT116 counterparts. SW480s showed signs of recovery following 5FU treatment such as: increased ER membrane proliferation and reduced ER distension (possibly linked to formation of autophagosomes), and increased Bcl2 expression, which could prove advantageous for drug resistance. In addition, TS induction following 5FU treatment was found to correlate with characteristic ER stress changes.

Conclusions: This study indicates that induction of TS by 5FU may cause ER stress and that a cell's endogenous adaptation to overcome this may determine their resistance. ER stress could cause release of damage associated molecular patterns and subsequent immunogenic cell death if the immune system is intact.

P12

EREG and DUSP6 protein expression and RAS mutational status in colorectal cancer

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Purpose: Numerous members of the MAPK signalling pathway have been implicated in the pathogenesis of colorectal cancer. EREG, a ligand of the epidermal growth factor receptor and DUSP6 a negative regulator of ERK1/2 are two such markers. In this study we aimed to assess the expression of EREG and DUPS6 at protein level in primary CRC resections and interrogate their utility as prognostic markers.

Methods: We assessed EREG and DUSP6 protein expression through multiplex immunofluorescence, quantified using the AQUA system on a cohort of 526 consecutively collected primary FFPE colorectal cancer resections representing all stages. All statistics were corrected for false discovery rate. Follow up clinical data was available for up to 4 years.

Summary of Results: Statistical analysis of expression of biomarker immunofluorescence demonstrated that for the whole study cohort low EREG (3yr; p <.001, 4yr; p .024) and low DUSP6 expression (3yr; p .001, 4yr; p .006) were independent biomarkers and in combination were associated with a significantly poorer survival outcome (3yr; p .001, 4yr; p .024). In sub-group analysis using clinicopathological and genotypic parameters a poorer survival outcome was identified in RAS wildtypes for low EREG (3yr; p .004, 4yr; p .032) and DUSP6 expression (3yr; p .001). Combinatory EREG/DUSP6 expression was significantly prognostic for both RAS wildtype and mutants at 3yr endpoint (RAS wildtype; p .041, RAS mutant; p .008) but RAS mutant only at 4yr endpoint (p .024). Interestingly, DUSP6 expression was associated with tumour site, rectal tumours having a higher proportion of low DUSP6 expression compared with right sided tumours (p .002). A significantly higher proportion of BRAF mutant individuals (97.9% of mutant population) had high DUSP6 expression compared with wildtypes (p .006).

Conclusion: DUSP6 and EREG may be related to prognosis in CRC, but further work is required to establish any possible mechanistic basis.

P13

Whole Exome Sequencing-Based Mutation Identification for Patient-Specific ddPCR Assays to Monitor Circulating Bladder Tumour DNA

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Purpose of the Study: Bladder Cancer (BC) is high recurrent and regular monitoring by invasive cystoscopy is a burden. Detection of circulating tumour DNA (ctDNA) in liquid biopsies is non-invasive, and may identify a relapse earlier. While ctDNA-based biomarkers should be technically feasible, an overall lack of common mutations is a challenge in BC. The aim of this study was to evaluate the feasibility of droplet digital PCR (ddPCR) assays in the context of BC and to investigate whether Next Generation Sequencing-based approach could be used to identify mutation to produce patient-specific digital droplet PCR (ddPCR) assays.
Methods: Samples were collected under MI84 ECMC Blood Biomarkers Study. Matched FFPE tumour samples were obtained from NHS GGC Biorepository. Whole Exome Sequencing (WES) was performed using Illumina NextSeq 500 system.
Summary of Results: A PI3KCA E542K and TP53 Y163C mutation were identified in tumours by SNaPshot and Sanger sequencing, respectively. ctDNA in patient's plasma and urine were successfully monitored by ddPCR in two cases of muscle invasive BC. WES of pT2G3 BC tumours (n=3) identified 19674-39776 tumour-specific mutations. There were no common mutations among three tumours. Mutations from the three areas within one tumour contained 19618-21525 mutations, of which 142 were common. CTNAP4 G727* mutation was selected from the pool and the mutation was confirmed by Sanger sequencing. A designed ddPCR assay was able to detect the mutation in the tumour DNA.
Conclusions: Using pre-validated, commercially available ddPCR assays, ctDNA could be monitored in both plasma and urine from BC patients, however availabilities of the assays are limited. Tumour mutations could be identified from patients' FFPE tissues by WES and subsequently ddPCR assays could be designed. Tumour heterogeneity would remain as a challenge, as WES is unlikely to provide any advantage in finding common assay targets.
This work has been funded by the Pathological Society.

P14

Importance of Combined MSI Testing and MMR Immunohistochemistry for Lynch/HNPCC on Colorectal Cancer Specimens and Correlation with 2017 NICE Guidelines: A Tertiary Centre Study

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Purpose of the Study: Lynch syndrome (LS) is a hereditary condition associated with a high risk for colorectal cancer (CRC) and is caused by germline mutations in DNA mismatch repair (MMR) genes. The two screening tests used in LS prediction are MMR protein expression assessed by immunohistochemistry (IHC) and DNA microsatellite instability (MSI) analysis. Previously accepted knowledge had been that MSI was superior to IHC in predicting a germline mutation. However, later studies showed that whilst both had limitations, MSI was more sensitive than IHC but slightly less specific.
Methods: We compared IHC and MSI results retrospectively from preselected CRC cases (<50 years / family history and histomorphological criteria suggestive of LS) tested between 2013-2017 to ascertain the presence and level of discrepancy between the two methods.
Summary of Results: 516 CRCs were analysed. Out of 419 which were MMR proficient, 333 (79%) were MS-stable, 2 were MSI-indeterminate (one due to suboptimal DNA quality), 3 were MSI-high, 8 were likely stable or stable but technically suboptimal, and for 8 specimens MSI testing was unsuccessful. In the remaining 65 cases MSI testing was not performed. MSI analysis had also been performed on 25 of the 97 samples showing abnormal MMR immunohistochemistry: 20 specimens were MSI-high (80%), 4 were MS-stable and one was likely to be MSI-high.
Conclusions: There are discrepancies between MMR and MSI results. Though this is a rare event, performing either test alone may result in patients who warrant further investigation for germline mutations being missed. Furthermore, testing on aged FFPE samples can result in limitations in PCR success due to impaired DNA quality. Although aged specimens often show a retained MMR IHC pattern, MMR IHC performance can be affected by prolonged storage. The failure of successful MSI testing, especially on an aged specimen, should therefore result in routine genetic discussion regardless of MMR IHC status.

P15

SDH-C Epimutated Gastrointestinal Stromal Tumours: Clinical and Pathological Features and SDH-C Promoter Methylation Analysis

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Background: Gastrointestinal stromal tumours (GIST) are the most common sarcomas of the gastrointestinal tract. Whereas the majority of GIST harbour mutations in the KIT, PDGFRA or BRAF genes, a subset referred to as 'wild type' (WT)-GIST have germline or somatic mutations and epimutations in genes encoding the succinate dehydrogenase (SDH) complex or occur in association with hereditary syndromes such as neurofibromatosis type 1. Here we present selected cases form the national wild-type GIST clinic with the rare finding of an SDH-C epimutations. We also present results on SDH-C promoter methylation analysis by pyrosequencing.
Methods: All patients have consented for molecular and germline testing and are taking part at the national WT-GIST clinic. Histology, SDH-B immunohistochemistry and somatic molecular testing was reassessed or completed. Somatic mutation analysis was performed by NGS using the Ion Torrent IonAmpliSeqTM Cancer Hotspot Panel v2. SDH-C promoter methylation was analysed by an in-house pyrosequencing assay.
Results: SDH-C epimutated patients are predominantly young females with primary WT-GIST of the stomach and negative for SDHB by immunohistochemistry. All tumours showed immunohistochemical loss of SDH-B protein, suggesting deficiency of the SDH complex. Germline genetic analysis did not identify a pathogenic mutation in SDHA/SDHB/SDHC and SDHD. The tumours showed SDH-C promoter hypermethylation.
Conclusions: SDH-C epimutated GIST account for a minority of all GISTs and occur mostly in young women with gastric primary. SDH-C promoter methylation testing is a sensitive method for further sub classification of SDHB deficieny WT-GIST. This offers important prognostic information as somatic SDHC epimutation is associated with higher risk of metastatic disease but is also associated with the development of additional tumours such as paraganglioma and pulmonary chondromas.

P16

BRAF Mutation Testing in Melanoma Using Pyrosequencing, Idylla™ and Cobas a Comparative Study

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Fast and accurate diagnostic systems are needed for the delivery of effective treatment in patients with rapidly progressing advanced melanoma. This study aims to compare the sensitivity, specificity and concordance of pyrosequencing, Idylla™ and cobas platforms for BRAF V600 mutation testing in melanoma samples. Cobas and Idylla™ tests are CE-IVD marked real-time PCR-based assays designed to detect the presence of the BRAF V600 mutation in FFPE specimens. BRAF inhibitors have been demonstrated to be effective in the treatment of metastatic melanomas carrying a V600 mutation; hence the requirement for a BRAF V600 mutation test. This study is the first large study comparing these platforms in melanoma samples. The cobas, Idylla™ and pyrosequencing platforms are all comparable systems to use for molecular diagnostics. This study demonstrates that the fully automated molecular diagnostics system Idylla™ is a promising technology for BRAF testing in melanoma. Idylla™ has increased sensitivity for rarer (non-V600E) mutations as compared to cobas, but pyrosequencing is more accurate overall in the detection of rare BRAF mutations (including codon 601). Missing these variant mutations would exclude a subset of patients from potentially effective BRAF or MEK targeting therapies. However, due to the high repeat rate of pyrosequencing and a difference in testing costs we recommend the use of multiple technologies to maximise the number of patients getting access to targeted therapies.

P17

Mismatch Repair Protein Deficiency and Colorectal Neuroendocrine Tumours

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Loss of DNA mismatch repair (MMR) protein expression is known to lead to cancer formation in hereditary non-polyposis colorectal cancer (HNPCC) also known as Lynch Syndrome, as well as some other sporadic colon cancer cases. The NICE guidelines recommend the testing of all colorectal cancer patients to maximise detection of Lynch families. In NHS Lothian, the testing protocol includes mismatch repair immunohistochemistry (MMR IHC) and microsatellite instability for samples with an equivocal or failed MMR IHC result. A BRAF test is employed for tumours with loss of MLH1 and PMS2 to determine if the defect detected is sporadic in type. A small proportion of colorectal tumours are neuroendocrine in phenotype. MMR protein loss is not well studied in neuroendocrine tumours (NETs) of colorectal origin, and so the objective of this study is to determine whether MMR loss is observed in colorectal NETs as this would help with the selection criteria for Lynch syndrome screening. Thirty seven patients (18 male, 19 female) were identified with NETs of colorectal primary origin (cNETs). MMR protein expression was assessed by immunohistochemical staining for MLH1, MSH2, MSH6, and PMS2. Positive staining for all four proteins was seen in all 37 cNETs. In this cohort, MMR protein deficiencies were not seen, suggesting that this pathway may not play a role in the pathogenesis of cNETs.

P18

Novel CAMTA1-FGF12 Translocation Identified in a Synovial Sarcoma

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Around 20% of sarcomas have simple karyotipic abnormalities and approximately 70 chromosomal translocations can be used for diagnosing 35 sarcoma types. Identifying specific translocations provides clarity for diagnosis and downstream management, and the absence or presence of a translocation may also have a prognostic value. The use of NGS panels in this clinical setting helps identifying well – characterised translocations for which no FISH probes are currently available and it also allows the identification of novel uncharacterised fusions. Synovial sarcoma is a rare malignant soft tissue tumour which accounts for 5–10% of soft tissue sarcomas. The defining feature of these tumours is the t(X;18) translocation. This translocation occurs when the SS18 gene on chromosome 18, fuses with the SSX gene on chromosome X, resulting in a chimeric transfusion protein transcript (from either SS18-SSX1 or SS18-SSX2). Synovial sarcomas are commonly found on the extremities around joints and tendons such as the knee, and often metastasize to the lymph nodes or lung. We report a novel CAMTA1-FGF12 fusion identified in a synovial sarcoma whilst assessing the viability of using NGS to identify sarcoma translocations with the TruSight RNA Fusion panel (Illumina). This fusion is a novel finding of unknown significance which may have diagnostic and therapeutic implications for the management of synovial sarcomas.

P19

The Genomic Landscape of Undifferentiated Pleomorphic Sarcoma

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Introduction: High grade undifferentiated pleomorphic sarcomas (UPS) of soft tissue are highly aggressive tumours with no specific lines of differentiation. Their pathogenesis is poorly understood which has led to significant challenges in diagnosis and therapeutic management. In view of the lack of objective diagnostic criteria and treatment options, there is an urgent unmet need to develop a molecular classification of the disease in order to stratify these patients for suitable clinical trials. Comprehensive genomic profiling is also likely to provide critical insight into the evolutionary pathobiology of these enigmatic tumours.
Aim and Objective: Molecular characterisation of UPS through comprehensive genomic profiling.
Results: We performed whole genome sequencing of 70 primary sarcomas pathologically classified as UPS or spindle cell sarcoma (not otherwise specified). Here we describe that up to 10% of these sarcomas have a hypermutator phenotype mostly underpinned by mismatch repair gene mutations. There is also significant enrichment of mutations and copy number alterations in genes involved in maintaining genome stability, cell cycle regulation, and telomere maintenance. This data is correlated with mutational phenomena such as chromothripsis, kataegis and whole genome doubling thus providing insights into tumour development and evolution. Importantly mutations are seen in druggable pathways such as mTOR signalling. There is also evidence that whole genome profiling has important diagnostic utility in this nebulous tumour type by identifying cases that had been misclassified by histopathology alone. This has important implications for pathologists using whole genome sequencing as a diagnostic tool in the present genomic era and importantly provides a biological rationale for objective clinical trial recruitment.

P20

Routine Use of DNA Methylation Arrays in the Diagnosis of Paediatric Brain Tumours in a Specialist Centre

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Primary CNS tumours are the most common solid malignancy in children and the commonest tumour-related cause of death. Amongst survivors, long-term disability is common. Therefore, accurate diagnosis is essential to focus aggressive treatments on the children who require it and avoid potentially damaging treatment in those that do not require it. DNA methylation provides data about tumour subtype and can be used to identify copy number changes. Several studies in a research context have indicated that methylation profiling may provide an important adjunct to conventional pathological diagnosis. In this study, we assessed the impact in a clinical service of methylation profiling for childhood brain tumours. The histology, molecular and methylation array results for over 150 cases of primary CNS tumours were reviewed from a specialist paediatric centre. The cases were divided into two groups: cases which reached a confident provisional diagnosis using routine diagnostic methods without methylation array data (Group A), and cases which did not (Group B). A "confident diagnosis" was defined as an unequivocal diagnosis recognised in the WHO Classification of Tumours of the CNS (2016 edition). Illumina EPIC arrays were undertaken on DNA extracted formalin-fixed paraffin embedded tissue. The provisional diagnosis made via routine methods was compared with the methylation array output and final diagnosis. The impact and clinical utility of the classifier on the final diagnosis was assessed in each case. Methylation profiling had a significant impact on diagnosis in group B cases and in subtyping in group A cases. Methylation array profiling shows promising results in CNS tumour identification and subtyping in paediatric clinical practice. Our data indicates that it can be incorporated in to routine practice in a clinical service.

P21

Skeletal Metastases of Papillary Thyroid Carcinoma – Report of Two Cases

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Metastasis to regional lymph nodes is the most common spread of papillary thyroid carcinoma (PTC). Contrary to follicular carcinoma, skeletal metastases by PTC are uncommon with occasional reports in the ribs, pelvis, vertebra, skull, humerus, and femur. Here we report two cases of PTC with metastatic deposit in the femur bone (case1) and to the skull, right breast, pancreas, and hip bone (case 2). In case 2 biopsy taken from the right breast showed the same histological features of the primary site resected in 2012 and reported as widely invasive PTC with tall cell component. Patient 2 was staged as pT3 N1 for the presence of three, synchronous, lymph node metastases. No biopsy was taken from other metastatic sites because the Radioactive Iodine (RAI) uptake was considered adequate evidence of metastases. Following radio-iodine ablation and high dose palliative radiotherapy the patient is alive with disease 5 years post thyroidectomy. Our finding suggest that PTC can have haematogenous spread and widely invasive PTC with a tall cell component are particularly prone to this type of metastatic spread.

P22

Follicular Dendritic Cell Sarcoma Presenting as a Parotid Neoplasm

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FDCS is a type of sarcoma of low to intermediate malignant potential, originating from follicular dendritic cells, which are non-lymphoid and non-phagocytic accessory cells of the lymphoid system. FDCS is a very rare neoplasm with 129 cases reported in the literature, including 67 cases in the head and neck. It affects males and females equally and at all ages with a mean age at presentation of 46 years. Approximately two thirds arise in a lymph node and one third arises in extra nodal sites, including the head and neck, oral cavity, GI tract, liver and spleen. We present a case of follicular dendritic cell sarcoma involving the parotid gland in a 51 year old female presenting as a painless mass. The patient underwent core biopsy which was interpreted as consistent with squamous cell carcinoma (SCC). The subsequent superficial parotidectomy showed an intraparotid lymph node replaced by proliferation of spindle shaped cells with atypical nuclei and mitoses. The storiform and whorled pattern with background inflammatory cells was unusual for SCC and immunohistochemistry was performed. The cells were positive for CD21, focally positive for CD23 and CD68. They were negative for CD45, p63, SMA, ER, PR, AE1/AE3, CD1a, S100 and Cam 5.2. Androgen receptor shows weak nuclear positivity. The proliferation index was 30-40% which led to the histological diagnosis of follicular dendritic cell sarcoma. Review of the literature showed only two cases of follicular dendritic cell sarcoma presenting as a parotid neoplasm. The present case confirms the difficulties to achieve a pre-operative diagnosis and a possible misdiagnosis such as squamous cell carcinoma requiring a different patient management. SCC tends to be treated with radio/chemotherapy while radical surgery is curative in up to two thirds of cases of FDCS.

P23

Intraductal Papillary Mucinous Neoplasms in Pancreatic Resections

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Intraductal papillary mucinous neoplasms (IPMN) are mucinous, often cystic, lesions of the pancreas usually occurring along the main pancreatic duct. They may be incidentally detected on radiology or, through mucus plugging of ducts, may cause chronic pancreatitis. IPMN are classified into gastric, intestinal, pancreaticobiliary, oncocytic subtypes, but data from studies on the prognostic significance of these are mixed. Some contain varying degrees of dysplasia and they may be associated with increased risk of pancreatic carcinoma. Increasingly, pancreatectomy is performed when IPMN is detected. The aim of this study is characterise the nature of and association with malignancy of IPMN in pancreatic resections. We identified all cases of resections where IPMN was present from 2009 to July 2017. The patient's age, subtype of IPMN, grade of dysplasia, associated malignancy and use of immunohistochemistry were recorded for all cases. 46 resection specimens were received. The average age of patients was 64.9 (standard deviation: 10.1). 50% were partial pancreatectomies, 11% were total and 39% were pancreaticoduodenectomies. 31 specimens were subtyped; 18 were gastric, 5 were intestinal, 5 showed gastric and intestinal elements and 3 were pancreaticobiliary. Immunohistochemistry was performed for confirmation of type in 27 cases. Overall, 27 contained low-grade dysplasia, 4 moderate and 8 high-grade. In 10 cases, Ki67 immunohistochemistry was performed. 22 IPMN were associated with malignancy (16 pancreatic adenocarcinoma, 4 neuroendocrine, 2 cholangiocarcinoma). 41% of malignant cases were pT2, 59% were pT3 and 68% had local nodal metastases. Subtype did not predict risk of malignancy. This study provides further evidence of the association between IPMN and pancreatic malignancy. In further studies it would be worthwhile to delineate the molecular biology of IPMN and associated dysplasia, and establish the events associated with the progression to malignancy.

P24

Cytological Assessment of Intraductal Papillary Mucinous Neoplasms of the Pancreas

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Intraductal papillary mucinous neoplasms (IPMN) have been increasing in incidence in the last decade, which may be partly explained by better recognition, more sensitive radiological techniques and an increased tendency to sample them. Although most IPMN contain at most low-grade dysplasia, some show high-grade dysplasia and they are occasionally associated with carcinoma. Therefore, sampling by fine needle aspiration for cytological assessment is undertaken to identify their potential for malignant transformation. The aim of this study is to establish the frequency of IPMN and dysplasia in a series where cytology is undertaken for clinically suspected IPMN. We identified all cases of cytology from 2009 to July 2017 where the clinical indication was IPMN or a mucinous cyst. The clinical indication, patient's age, final diagnosis, grade of dysplasia and use of immunohistochemistry were recorded for all cases. 74 cases for cytology meeting criteria were received over an 8 year period. The average age of the patients was 68.6 (standard deviation: 11.3). In 72% of cases, there was a suspicion of IPMN; in 24% the history was of a cyst; in 4%, IPMN with associated adenocarcinoma was suspected. In 29 cases IPMN was listed as a differential diagnosis. 17 cases were non-diagnostic, 8 cases were described as a cyst without further detail, 13 were normal/benign without further detail, 4 were atypical and 3 were definitely malignant, one of which was associated with IPMN. Of the IPMN cases, 8 had no dysplasia, 8 had "no high-grade dysplasia", 7 had low-grade dysplasia, 2 had moderate dysplasia and 4 had high-grade dysplasia. 7 cases were supplemented with immunohistochemistry, in particular Ki67. This study demonstrates that cytological assessment is a valuable technique for the identification of risk of malignancy in IPMN to guide further therapy. Improving the adequacy of specimens would allow further investigation by immunohistochemistry to support the diagnostic process.

P25

Immunohistochemical Characterisation of Inflamed and Histologically Normal Gallbladders

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Introduction: We aimed to establish features of inflammation in histologically normal gallbladders with gallstones and compare the expression of inflammatory markers in acutely and chronically inflamed gallbladders.
Methods: We studied four groups of patients: Group I - chronic cholecystitis (n=60), Group II - acute cholecystitis (n=57), Group - III histologically normal gallbladder with gallstones (n=45) and Group IV - incidental cholecystectomy at hepatectomy/pancreatectomy (n=60) after reviewing the histology of H&E stained sections. Immunohistochemistry was performed for IL-2R, IL-6, TNF-a and Substance P. The immunostaining was assessed quantitatively/semi-quantitatively in full-face sections.
Results: Median, Q1-Q3 mucosal IL-2R expression in Groups I (2.65, 0.87-7.97), II (12.30, 6.15-25.55) and III (0.40, 0.10-1.35) was increased compared with Group IV (0.25, 0.10-0.50, $p<0.05$). Submucosal IL-2R expression in Group I (2.0, 1.12-4.95), II (10.0, 5.95-14.30), III (0.50, 0.15-1.05) was also increased compared with IV (0.10, 0-0.30). Lymphoid cell IL-6 expression in Groups I (5.95, 1.60-18.15), II (6.10, 1.1-36.15), III (8.30, 2.60-26.35) was increased compared with Group IV (2.50, 0.60-4.45, $p<0.05$). Epithelial TNF-a expression in Groups III (85.0, 70.50-92.0) and IV (83.5, 65.75-91.75) was increased compared with Groups I (72.50, 45.25.0-85.50) and II (61.0, 30.0-92.0, $p<0.05$). Lymphoid cell substance P expression in Groups I (1.90, 1.32-2.65), II (5.62, 2.50-20.8), III (1.0,1.0-1.30) was increased compared with Group IV (0.85, 0.50-1.0, $p<0.05$). White Cell Count in Group II (10.0, 7.20-13.50, $p<0.05$) was increased compared with Groups I (7.70, 6.30-9.90), III (6.95, 5.85-8.35), IV (7.15, 6.30-8.67).
Conclusion: Histologically normal gallbladders with gallstones exhibit features of inflammation that cannot be detected with conventional histological examination. Therefore, there may be a case of offering cholecystectomy to patients with asymptomatic gallstones.

P26

Groove Pancreatitis Associated with Real and Apparent Neoplastic Change

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The pancreatic groove area, between duodenum, bile duct and pancreatic head, usually lacks pancreatic tissue and is composed of fibrous connective tissue with neurovascular bundles. Occasionally, heterotopic pancreatic tissue exists here, with variable relationship to the layers of the duodenal wall. Inflammation and malignancy of this tissue has been described. We present a series of groove pathologies and report two conditions; groove neuroendocrine carcinoma; and groove carcinoma in-situ. A retrospective review was undertaken of cases of groove pathology identified from pancreaticoduodenectomy (PD) specimens in a single centre between 2011 and 2016, assessing preoperative investigations, histological details and outcomes. Groove pathology was found in six patients having PD for presumed cancer (4 male, 2 female, median age 58 y, range 47-78 y). All had histological evidence of groove pancreatitis. Two patients had neoplastic change within groove heterotopia; one had a synchronous groove neuroendocrine tumour and one had groove carcinoma in-situ (PaIEN G3) in dilated heterotopic ducts diagnosed after PD for pancreatic ductal adenocarcinoma (PDAC). In two patients, groove pancreatitis accompanied other neoplasms; one PDAC and one intraductal pancreatic mucinous neoplasia. Finally, in two patients presenting with jaundice, groove pancreatitis was the only pathology. Both had PD for presumed cancer with preoperative brush cytological highly suspicious of malignancy. All six had preoperative CT pancreas and EUS and/or ERCP; none diagnosed groove pathology. Pancreatic groove carcinoma in-situ and neuroendocrine carcinoma are newly reported conditions. Pancreatitis may be seen associated with other pancreatic neoplasia; it is not well assessed with conventional cross sectional imaging and may provide brush cytology highly suspicious for malignancy. PD offers the best prospect of definitive management of groove pathologies.

P27

Cytological and Histological Assessment of Pancreatic Lesions in a Tertiary Hepatobiliary Unit

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Introduction: Whilst there has been a steady improvement in mortality rates for patients with pancreatic cancer, it still remains the fifth most common cause of cancer death in the UK. With late diagnosis of the disease and substantial post-operative morbidity rates, the role of cytology in pancreatic lesions has been on the incline. The current study aims to assess correlation between pancreatic histological and cytological specimens, and respective turnaround times.
Methods: All patients undergoing pancreatic Fine Needle Aspiration (FNA), biopsies and resections between September 2015 and 2016 were identified from a WinPath database. Dates of specimen reception and authorisation were noted, and both reports were analysed for concordance.
Results: Out of 208 patients, 234 pancreatic FNAs were performed, and thirty five patients (16%) also had histological reports (either a core biopsy or a resection). Eighty percent of cytology was reported within 7 days, 78% of core biopsies and 42% of histology within 10 days. A 74% correlation was present between cytology and histology reports. The vast majority of non-correlating cases were due to non-representative cytology specimens, as commented upon on the reports.
Conclusion: This audit has highlighted good correlation between histological and cytological findings. Non-concordance was mostly due to non-representative cytology specimens. Recent literature shows usage of a larger bore needle and high negative pressure when sampling may increase adequacy of cytological specimens. Factors influencing turnaround times will be assessed prior to re-auditing.

P28

In Situ Mutation Detection Maps the Clonal Architecture of Colorectal Cancer and Adenomas

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Current methods to measure intra-tumour heterogeneity, such as genome sequencing, do not preserve spatial information and consequently the relationship between mutant clones and their histopathological context is lost. To address this shortfall, we have developed and validated BaseScope, a novel mutation-specific RNA in situ hybridization assay. We use BaseScope to map the spatial and morphological context of subclones in colorectal adenomas and cancers that bear common driver point mutations in KRAS, BRAF and PIK3CA genes. Computational modelling of these spatial-genetic data showed that subclones must have arisen sufficiently early, or have a considerable fitness advantage, to form large or spatially disparate subclones. The BaseScope assay was able to detect even very minor KRAS mutant subclones, and also detect KRAS subclones that were localised to the adenomatous compartment of mixed lesions, with potential ramifications for anti-EGFR treatment decision making. BaseScope represents a significant advance for in situ mutation detection that provides new insight into tumour evolution, and could directly assist histopathological diagnosis and prognosis.

P29

Poorly Differentiated Clusters as a Single Marker are not Superior to Tumour Budding in Predicting the Need for Major Resection in Early Colorectal Cancers (CRC)

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Introduction: Detection of early colorectal cancer lesions through implementation of the National Health Service Bowel Cancer Screening programme has increased pT1 CRC's three fold to 17%, but how to manage them is still uncertain. Recently tumour budding (TB) and poorly differentiated (PDC) have emerged as new reproducible quantitative markers to predict lymph node metastases (LNM). We compared their ability to predict LNM in 206 symptomatic patients with pT1 colorectal cancer (19 with LNM).
Method: Quantitative markers of TB and PDC were measured as previously described. The modified ROC curve values with the highest sensitivity and specificity in predicting LNM for TB led to a cut-off of 2 and for PDC of 8. Further the data was evaluated with the cut-off values in keeping with the Japanese classification of PDC <5 (G1), 5 to 9 (G2), and > 10 (G3). Associations between categorical data and LNM were performed with the X2 and Fisher's exact tests.
Results: A higher number of TB significantly predicted LNM in CRC (p=0.007). TB could identify 16/19 (84.2%) of LNM thus indicating resection in 55.3% (114/206). PDC with a cut-off of 8 was significant to predict LNM (p=0.042). PDC could identify only 8/19 (42.1%) of LNM and would lead to a resection rate of 22.3% (46/206). PDC classified according to the Japanese classification was not significant to predict LNM. PDC could identify in the G3 high risk group 5/19 (26.3%) of LNM cases (25/206; 12.1%), in the intermediate G2 group 6/19 (31.6%) of LNM cases (67/206; 32.5%) and in the G1 group 8/19 (42.1%) of LNM cases (114/206; 55.4%).
Discussion: Although recent data suggested that PDC are superior to TB in predicting LNM in Japan we could not verify this in our cohort with our ROC cut-offs nor those of the Japanese guidelines. With our ROC derived cut-offs we identified only 42.1% of LNM cases with PDC in comparison with 84.2% with TB. We are currently investigations TB and PDC in a bigger cohort.

P30

Examining the Enteric Bacterial Metagenome in Comorbid Inflammatory Bowel Disease and Irritable Bowel Syndrome

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Purpose of the Study: A significant proportion of inflammatory bowel disease (IBD) patients experience symptoms akin to irritable bowel syndrome (IBS) without mucosal inflammation, and have a quality of life that is not significantly different from those with active IBD. The gastrointestinal microbiome may play a role in IBD and IBS, leading to speculation that altered floral composition or activity is responsible for their symptoms. A previous study has shown that no marked changes in composition are associated with these symptoms, but did not investigate the bacterial metagenome, which could provide insight into altered bacterial functionality in such patients.
Methods: Stool samples were collected from patients with CD (n=150) and UC (n=120). The presence of inflammatory activity was determined using faecal calprotectin levels. Four disease status groups were defined - 'true IBS', 'active IBD', 'quiescent IBD', and 'occult inflammation'. 16S rRNA sequencing data was used to infer the bacterial metagenome. ***Work was supported by a PathSoc Grant.***
Summary of Results: Despite marked variation in microbiome composition, inferred bacterial metagenomes were similar. One orthologue, responsible for the bacterial phosphotransferase system (PTS), was significantly differentially abundant across the groups (p=0.034).
Conclusions: The results of this study provide further evidence that the persistent IBS-type symptoms seen in quiescent IBD patients are not the result of changes in the composition or activity of the gut flora. The relative lack of variation in bacterial metagenome composition compared to marked variation in microbiome composition suggests that researchers investigating the microbiome should be more conservative in their interpretations of the relevance of changes in the abundance of bacterial taxa, as these may not correspond to functional differences.

P31

The Role of Radiological, Biochemical and Cytopathological Findings of EUS-FNA Techniques in the Assessment and Diagnosis of Pancreatic Cystic Lesions: A Single-Centre Diagnostic Test Accuracy Study

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Purpose of Study: Pancreatic cystic lesions are an increasingly detected heterogenous group of histopathologic entities with varying degrees of clinical significance, malignancy and outcome. EUS-FNA is a diagnostic tool providing high-resolution imaging of pancreatic cysts, and aspiration of cystic fluid for biochemical and cytological analysis. Existing literature provides varied reports of the sensitivity and specificity of these techniques. This study aims to provide evidence to aid future evaluation of these techniques.
Methods: This study was retrospectively performed by consulting the UHB pathology database (Telepath) to identify all cases coded as a pancreatic EUS-FNA taking place between 01/01/14 and 31/12/16. Cases that had undergone a corresponding resection were then identified. UHB clinical portal was used to manually code cases for biochemistry and cytological features. Analysed groups were based on histological diagnosis following resection if available, and MDT verdict if not. Mann-Whitney U-test was used to assess statistical significance.
Results: A total of 227 cysts were identified, including 90 intraductal papillary mucinous neoplasms (IPMNs), 6 mucinous cystic neoplasms (MCN), 29 serous cystadenomas, and 41 pseudocysts. Combined cytological and biochemical analysis was reliable in differentiating mucinous from non-mucinous lesions. Low-grade IPMNs had significantly lower levels of CEA compared to other mucinous lesions. Amylase was not significantly different. Higher grade IPMN had less extracellular mucin than low-grade IPMN and MCN. MCN were more likely to be acellular than IPMNs.
Conclusion: Combined cytological and biochemical analysis is valuable in the work up of patients with pancreatic cysts. In mucinous lesions the amount of mucin and the CEA level varied according to the degree of atypia. Mucinous cystic neoplasms may be more likely to give an acellular sample than other mucinous cysts.
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P32

Identification of Optimal Immunohistochemical Parameters to Separate Coeliac Disease from Normal Duodenal Biopsies, with Implications for Digital Image Analysis Development

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Coeliac disease (CD) has around 1% prevalence and is due to an immune response to gluten that damages the small intestine. Manifestations range from no symptoms, through anaemia to severe intestinal symptoms, with complications (bone thinning, infertility, lymphoma and duodenal cancer). Other gluten-sensitive conditions (GSC), including non-coeliac gluten sensitivity, irritable bowel syndrome and wheat allergy, fail to fulfil CD criteria, but are up to 12 times as common. The exact relationship between these conditions remains unclear. Treatment of CD is a lifelong gluten-free diet (GFD), but importance of GFD is unclear in other GSC. We hypothesise that more objective and accurate CD diagnosis might be achieved by digital image analysis, either alone, or as part of multiparameter clinical and laboratory analysis.
We set out to determine which two routine immunohistochemical (IHC) stains best separate CD (n=10, Modified Marsh Score grade 3a, 3b or 3c) and normal duodenal biopsies (n=10). Sections were immunostained and parameters evaluated manually, with differences between CD and normal as follows: intraepithelial lymphocytes per unit volume: CD3+ ($p<2.1 \times 10^{-6}$), CD4+ ($p=0.26$) and CD8+ ($p<1.4 \times 10^{-7}$); lamina propria lymphocytes per unit volume CD3+ ($p=0.15$), CD4+ ($p=0.13$) and CD8+ ($p<1.4 \times 10^{-7}$); percentage Mib1 expression in the lowest 50 epithelial cells each side of crypts ($p<0.002$).
We conclude that, when developing a deep learning analytical algorithm for digital image analysis, provided that our algorithm is able adequately to segment villous epithelium, lamina propria and crypt bases, the optimal IHC stains to accompany H&E are CD8 and mib1.

P33

Comparison of Pathological and Radiological Staging of Rectal Carcinoma

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Introduction: Magnetic Resonance Imaging (MRI) is the most accurate imaging modality for the local staging of rectal carcinoma. The highly detailed images obtained during T2-weighted, fast-spin echo (FSE) sequnces show a high degree of similarity to histopathology sections. Therefore, with careful and systematic interpretation, important supplementary information such as circumferential resection margin (CRM) involvement and extra-mural vascular invasion (EMVI) status can determine the degree of pre-operative therapy for patients and clinical outcome. In the absence of MRI staging, many of the prognostic variables would only be reliably determined through histopathological evaluation of the final operative specimen. Thus, clinicians may miss an opportunity to potentially downstage tumours and influence overall outcome.
Aim: The aims of this audit are to assess whether there are discrepancies between the MRI and pathological taging of rectal carcinoma – TNM staging, CRM involvement and EMVI status.
Target Criteria, Methods and Materials: 50 patients who had a confirmed rectal carcinoma over a 1 year period were identified with their staging by histopathology. The patients' MR rectum reports were then analysed through CRIS/ PACS radiologically, along with the past MDT staging.
Results: • STAGING. Tumour (T) staging discrepancy – 1/50 (2%) – T2 overcalled as T3, shown to be due to non-tumour related mesorectal stranding. • CRM INVOLVEMENT. 100% correlation between MR and histopathology. • EMVI STATUS. Discrepancy in 2/50 cases. One false negative and one false positive.
Conclusion: There is good correlation between histopathological and radiological staging of rectal carcinoma with regard to TNM staging, CRM involvement and EMVI status.

P34

This abstract has been withdrawn

P35

Mixed Neoplasms of Pancreas – A Review of Two Cases

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Pancreatic tumours are rare and could arise from either the exocrine (ductal and acinar cells) or the endocrine (neuroendocrine cells) components of the pancreas. In some instances, the occurrence of pancreatic tumors comprising both acinar cells and neuroendocrine cells, with neuroendocrine cells making up more than 30% of the tumor, has been identified. This unique entity has been referred to as mixed acinar-neuroendocrine carcinoma. Only about 30 such cases have been reported in the literature. Similarly tumours consisting of an adenocarcinoma component and a neuroendocrine carcinoma component, in which each component accounts for at least 30% of the tumour, are defined as mixed adenoneuroendocrine carcinomas (MANECs). MANEC of the pancreas is extremely rare, and the clinical behaviour remains unclear. We report two cases of mixed tumours of pancreas who presented with nonspecific complain. One of the patients underwent Whipple's resection and other underwent a distal pancreatectomy with splenectomy. The post-operative course and stay was uneventful. Macroscopic examination revealed mixed adeno-neuroendocrine tumour and mixed acinar- neuroendocrine carcinoma respectively. Mixed carcinomas of pancreas are rare and very few cases have reported in literature for which surgical resection remains the main treatment. The prognosis of these tumours is largely unknown. A correct identification of both components by morphology and immunohistochemistry is essential as many studies suggest that the prognosis is dependent on the endocrine component. Patients need regular follow-up since there remains much uncertainty with behaviour of these tumours.

P36

Role of PD-L1 in Gastrointestinal Solid Tumors

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Purpose: The aim of this study was to elucidate the roles of programmed cell death ligand 1 (PD-L1) in solid tumors of gastrointestinal tract.
Methods and Results: We investigated PD-L1 immunohistochemical expression in 57 NENs and 63 colorectal cancers and its correlation with grade, gender, primary site, histological type , lymph nodes status, MSI and peri-and intra-tumor immune cells (lymphocytes, macrophages and granulocytes). The PD-L1 positivity rate and signal intensity are directly correlated ($p< 0.001$) with grade increase, in particular from NENs G1 to NENs G3. Therefore, high grade tumors are characterized by significant PD-L1 expression in both the tumor and infiltrating immune cells ($p<0.001$), reflecting an unfavorable environment for T cell-mediated tumor aggression.
Conclusions: The immune escape mechanisms of GI tumors is not yet well characterized. The different kinds of GI neoplasms showed focal and heterogeneous PD-L1 expression on both tumor and immune cells than it is not possible performe this assay on bioptic samples. Our study provide evidence of strong PD-L1 tissue expression in gastroenteropancreatic neuroendocrine neoplasms and that the correlations with grade, MSI and histological type varied between different GI tumor. However, the prognostic role of PD-L1 remains controversial, because of natural tumor heterogeneity, variability in the assays, different histological grade/type, and cutoff values. The expected different PD-L1 expression in various systems of the human body (gastrointestinal, pulmonary, skin) need strict evaluation criteria for PD-L1 standardization. Moreover, PD-L1 might be a useful prognostic biomarker to approach immunotherapy treatment in solid GI tumor.

P37

A Case of Metastatic Melanoma Presenting with Ileocolic Intussusception 12 Years After the Excision of the Primary Skin Lesion

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Introduction: Recurrence from cutaneous melanoma can occasionally occur within the gastrointestinal tract; predominantly the small bowel.
Case: We describe the case of an 83 year old woman who presented acutely with an ileocolic intussusception due to a metastatic melanoma lesion. This occurred 12 years after excision of the initial melanocytic lesion, which had originally presented on the upper lip. She underwent an emergency right hemicolectomy as a result.
Discussion: Metastases from malignant melanoma to the gastrointestinal tract are not uncommon. The average timeframe between the diagnosis of the initial primary lesion, and disseminated disease presenting in the bowel, can be up to 54 months according to the literature. On the other hand, intussusception associated with melanoma is extremely rare. Both the manner in which this particular lesion presented, as well as the time period between the primary diagnosis and the occurrence of the metastases, makes this case particularly interesting and unique.
Conclusion: Patients who have a history of cutaneous malignant melanoma, presenting with vague or non-specific abdominal symptoms, should be investigated for bowel metastases, even in cases where there is a long disease-free interval.

P38

Collagenous Gastritis – An Interesting Case of this Lesser Known Entity

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A 49 year old female with a history of hypothyroidism and diabetes mellitus underwent gastroscopy as part of a pre-operative assessment prior to bariatric surgery. Endoscopic studies revealed multiple small gastric polyps and mild antral gastritis. Histologic examination of a biopsied polyp showed prominent sub-epithelial band like layer of hyalinisation of around 10-30 microns thick and an active inflammatory infiltrate, which stained positive for collagen on Masson's trichrome staining. Collagenous gastritis is a rare entity. It has been described in two groups of patients, paediatric and young adults. In the first group, symptoms and findings are mainly confined to the upper gastrointestinal tract, whilst the latter group present with diffuse involvement of the upper and lower gastrointestinal tract. Within the second subgroup, reported associations have included coeliac disease, lymphocytic gastritis, collagenous colitis, lymphocytic colitis and other autoimmune disorders. Whilst the pathogenesis remains unknown, several studies have suggested various causes including chronic inflammation, autoimmune response and primary vascular abnormality causing increased permeability. Clinical presentation varies from no symptoms to anaemia, epigastric pain, constipation and diarrhoea. Endoscopic findings typically include gastric erythema, erosions, haemorrhages and most commonly, mucosal nodularity which is found in both subgroups. Collagenous gastritis can be confidently identified by using analogous defined features of collagenous colitis; subepithelial collagen band of thickness greater than 10 microns, intraepithelial lymphocytes, lamina propria lymphoplasmacytic infiltrate and surface epithelial damage. Various treatment options have been trialled including histamine H2 receptor antagonists, sucralfate, furazolidone, prednisolone and elimination of gluten but no definite treatment has been identified.

P39

Frequency of Malignant Melanoma in the Gastrointestinal Tract

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Malignant melanoma is known to metastasise to the gastrointestinal tract, in particular to the liver. However, parts of the gastrointestinal tract such as the oesophagus and anus contain melanocytes, which may give rise to primary malignant melanoma. The aim of this study was to identify the frequency of malignant melanoma throughout the gastrointestinal tract and classify on the basis of primary or metastatic origin. We identified all cases of malignant melanoma between 2009 and July 2017 in the laboratory electronic reporting system using SNOMED identifiers to specify gastrointestinal organs, including salivary glands. We analysed the reports to identify whether the melanoma was of primary or metastatic origin. There were 54 cases of malignant melanoma arising in the gastrointestinal tract during the study period. Of these 43% were liver metastases. Primary malignant melanoma was present in 7 (13%) cases, of which 3 were anal, 3 were oesophageal and 1 arose in the parotid gland. Of the non-hepatic metastases, 8 occurred in the small bowel, 7 in the parotid, 5 in colon, 2 in the gallbladder and 1 in the stomach and in the pancreas. Our study demonstrates that malignant melanoma of the gastrointestinal tract is usually of metastatic origin. In most cases this should prompt thorough clinical and radiological assessment for the origin of the primary disease if this is unknown. However, in the anus and oesophagus, primary origin should be considered and indeed, albeit based on a limited dataset, primary origin is more likely than metastasis. The aetiology of these lesions is unclear and molecular characterisation in future studies would be beneficial in revealing the underlying aberrations which drive their behaviour.

P40

Effect of Hypervariable Region and Computerised Size Selection on Gastrointestinal Microbiome Data

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Introduction: The microbiome describes all micro-organisms within the human body. Altered gastrointestinal microbiome is associated with colorectal pathologies. Currently, choice of variable (V) region in 16S rRNA analysis results in bias towards particular bacteria in microbiome data. Our project aimed to explore this bias using simulated PCR and investigate methods, specifically "computerised size selection", that could reduce it.
Methods: DNA extraction, amplification and Next Generation Sequencing was carried out on 64 frozen faecal samples on regions v2, v3, v4, v5, v6 and v7/v8. Bioinformatic processing used python scripts, Taxman and QIIME software. "Computerised size selection" isolated amplified peaks of DNA that were outside the target base pair (bp) length of each primer. The bacteria identified in these peaks were analysed to see if they represented a true picture of gastrointestinal bacteria. For each primer we used the percentage prevalence of bacteria found by a simulated PCR of the entire greengenes database to score bias caused by V region selection.
Results: V3 and V4 were the least biased regions. However, due to all peaks being outside target size for the particular V3 primer we used, we can only recommend V4. We demonstrated that in all V regions "computerised size selection" could be used as a useful quality control mechanism. Once the aberrant peaks were removed this reduced percentage of unassigned bacteria without significantly affecting community correlation against non-trimmed data (p=<0.001).
Conclusions: Our results have reinforced the findings from previous literature that V4 gives the best coverage of the gastrointestinal microbiome. "Computerised size selection" was useful to obtain a more accurate picture of the bacteria identified by each primer.

P41

Neo-antigen Prediction in Bladder Cancer

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Purpose of Study: Tumour mutational burden and neo-antigen formation drive much of the immune response in melanoma and lung cancer and are associated with improved survival. To date, these factors have not been investigated in bladder cancer. We aimed to apply a neo-antigen prediction pipeline to bladder cancer data and investigate associations between neo antigen levels and clinical outcomes.
Methods: Whole exome and RNA-sequencing (WES and RNA-seq) data of bladder cancer cases from The Cancer Genome Atlas project were used. Twenty-nine frequently mutated genes (mutational frequency >3%) were used to construct potential 8-11 mer peptides in silico. These were filtered against known human peptides then assessed for MHC binding affinity using NetMHCpan. Neo antigen expression was confirmed in the RNA-seq data and associations with clinical variables then explored.
Summary of Results: Data were available for 131 patients. Neo antigens were identified in 12 patients prior to expression filtering and 3 patients after this step with one neo antigen per patient. No recurrent neo antigen producing mutations were identified. The low number of patients with neo antigens prohibited investigating any association with clinical outcomes.
Conclusions: We successfully applied our neo antigen prediction pipeline to bladder cancer cases. Invasive bladder cancer has low neo antigen production in a 29 gene panel production despite a high mutational burden possibly indicating deficient tumour immune response and/or immune escape of these tumours. Limitations included the small gene panel and lack of early stage cases. Further work will investigate a wider gene set, a larger cohort and non-invasive disease.

P42

Mucin Poor Mucinous Tubular Spindle Cell Carcinoma of the Kidney: Review and Report of Two Cases with Divergent Morphology; One Spindle Cell Predominant and One Having Cells with Signet Ring Morphology

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Background: Mucinous tubular spindle cell carcinoma (MTSRCC) is a rare low grade type of renal cell carcinoma (RCC).
Aim: Here we report two cases of MTSRCC that showed unusual and divergent morphology.
Summary of Results: Although both were mucin poor, the first case was spindle cell predominant with psammoma bodies, and thick walled blood vessels whereas the second one was tubular predominant with abundant clusters of foamy histocytes, cells with signet ring morphology, Fuhrman grade 3, and wide spread Neurospecific enolase (NSE) positivity. Diagnosis was confirmed by immunohistochemistry (CK7+, CD10-, SMA-, HMB45-, CD34-, CD15-, and CD117-). The presence of cells with signet ring morphology is the first time to be reported in MTSRCC.
Conclusions: It's possible that these two cases represent two different variants of MTSRCC and each of them has different features. Care should be taken when diagnosing a renal tumour with predominant spindle cell proliferation to avoid missing MTSRCC and confusion with other more aggressive types of RCC.
Keywords: Mucinous tubular spindle cell carcinoma. Renal cell carcinoma. Mucin poor. Psammoma. Signet ring. Fuhrman grade 3. Neurospecific enolase

P43

Granulomatous Tubulointerstitial Nephritis with Vasculitis

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Introduction: Granulomatous tubulointerstitial nephritis(GIN) comprises less than 1% of renal biopsies with a broad differential diagnosis including drugs, infections, and immune mediated diseases (sarcoidosis, tubulointerstitial nephritis with uveitis(TINU), granulomatosis with polyangitis(GPA) etc). We report two cases of GIN with vasculitis.
Case 1: A 44-year-old man presented with painful red eyes and deranged renal and liver functions. There was no history of drug intake, rash or respiratory symptoms. Serum Ca was elevated on one occasion. CECT of the chest and serum ACEI levels were normal. Gamma IFN release assay(IGRA) for TB, ANCA, ANA and dsDNA were negative. Renal biopsy showed GIN and granulomatous vasculitis. The patient is being managed as TINU with a favorable response to steroids.
Case 2: A 51-year man had elevated serum creatinine during follow-up after treatment for pneumonia. Serum Ca was elevated on one occasion with normal ACEI levels. IGRA and antibody screening were negative. Renal biopsy showed GIN and granulomatous vasculitis. CECT was negative for interstitial lung disease, but showed small non-calcific mediastinal and hilar lymph nodes. He continues to have cough, dyspnoea, blurring of vision, arthralgia and a rash. He has been given a presumptive diagnosis of sarcoidosis.
Discussion: Both cases showed granulomatous vasculitis. The absence of glomerulonephritis, negative ANCA serology, and morphology of the granulomas made GPA and infections unlikely. The lack of a temporal relationship with drug intake, and of systemic or interstitial eosinophilia made drugs an unlikely aetiology. TINU and sarcoidosis are the presumptive causes. Granulomatous vasculitis has been reported previously in only 4 cases of sarcoidosis and 1 case of TINU. These cases along with the literature review highlight that GIN with vascular involvement can be seen in GIN of a variety of causes and that careful clinicopathological correlation is needed to elucidate the aetiology.

P44

Snake Bite Nephropathy: A Case Series

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Introduction: Snake bite nephropathy(SBN) causes significant renal morbidity and mortality in Sri Lanka. This study aims to describe the renal histopathology of poor resolution SBN and outcome.
Methodology: Consecutive renal biopsies of SBN received at the Colombo University during a six month period were retrospectively studied using light microscopy.
Results: Eight biopsies from patients with poor resolution acute kidney injury (AKI) were studied. Biopsies were taken between 17-34 days following snake bite. All patients were previously healthy. The snakes implicated included 4 Hump nosed vipers, 1 Russell's viper, 1 unknown viper and 2 unidentified snakes. Cortical necrosis was seen in 2(25%), haemorrhagic glomerular necrosis in 3(37.5%) and mesangiolysis in 4(50%) cases. Ischaemic collapse of glomeruli, minor increases in mesangial matrix and cellularity were seen in all cases. One(12.5%) had focal segmental endocapillary hypercellularity with neutrophils. Five(62.5%) had segmental thickening of glomerular capillary basement membranes, two(25%) with segmental double contours. All cases had acute tubular injury, with necrosis in 5(62.7%). Red cell and granular casts were seen in all cases, with pigment casts in 7(87.5%). Interstitial oedema was present in all 8(100%) biopsies. Predominantly mononuclear inflammation was mild in 4(50%) and moderate in 4(50%). Tubular atrophy was minimal in 5(62.5%) and severe in 3(37.5%). Interstitial fibrosis was minimal in 2(25%), moderate in 2(25%) and severe in 4(50%). There was intimal thickening of blood vessels in 6(75%) and hyperplastic arteriolosclerosis in 1(12.5%). At follow up of 5 cases, 1 died, 3 progressed to chronic kidney disease and 1 recovered.
Discussion: A range of features attributable to ischaemic and toxic damage were seen, consistent with the literature. Mesangiolysis, previously only rarely described was seen in 50%. The outcome following prolonged AKI is poor with with 50% progressing to CKD in this series.

P45

Significant Ketoacidosis at Autopsy: A Single Centre Systematic Review

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Aim: To investigate the value of vitreous beta hydroxybutyrate and serum acetone in the investigation of sudden unexpected death.
Methods: Coroners' autopsy reports from a provincial UK city, population ~ 900,000, over a 24-month period, with significant ketoacidosis were studied. The city "continues to have deprivation hot spots that are amongst some of the most deprived areas in the country yet are adjacent to some of the least deprived areas in the country". Demographic features, medical history, anatomical and histological findings, biochemical parameters, including renal function, vitreous glucose, serum and vitreous alcohol were analysed.
Results: 42 cases (28 males and 14 females) were identified, 55% had a history of alcohol and/or substance misuse and mental health problems, particularly depression and anxiety. 16% were diabetics. Nearly 50% of subjects had alcoholic ketoacidosis, 17% had diabetic ketoacidosis and 10% had evidence of both diabetic and alcoholic ketoacidosis. In 8 cases (21%) no cause of ketoacidosis was established. In alcoholic ketoacidosis the subjects typically had low vitreous glucose and undetected alcohol levels. All of the subjects with raised vitreous glucose levels had diabetic ketoacidosis. In the majority of patients the heart weight was within the normal range (mean 374g, range 140-550g). Only two patients had heart weights > 460g. In two patients hypertension had been diagnosed in life and there was one case of alcoholic cardiomyopathy. In one patient cardiac failure was considered to be the primary cause of death and in two a cardiac arrhythmia was suspected. Ischaemic heart disease was not considered to be a cause of death in any patient.
Conclusion: Ketoacidosis is relatively common and should be considered as a cause of sudden death, especially in alcoholic and diabetic patients with no clear cause of death at autopsy.

P46

Cardiac Manifestations of Thrombotic Thrombocytopenic Purpura: A Four Case Study Series

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Objective: Thrombotic Thrombocytopenic Purpura (TTP) is a thrombotic microangiopathy characterised by a pentad of thrombocytopenia, macroangiopathic haemolytic anaemia, acute kidney injury, neurological dysfunction and fever.
Our objective was to identify cases of sudden death cases due to Thrombotic Thrombocytopenic Purpura referred to St George's University Hospital.
Methods: A retrospective search was made on referred material to the Cardiac Risk in the Young Cardiovascular Pathology unit at St George's University Hospital between 2000 to 2016. Criteria for search included cases with documented thrombocytopenia, anaemia and /or TTP related symptoms 72 hours prior to death. All died suddenly.
Results: Four cases were identified (Female; 33 to 66 years of age). Two cases showed epicardial haemorrhages with extension into the underlying myocardium. All four cases showed platelet rich thrombi in the cardiac microvasculature with microinfarcts.
Discussion: TTP incidence ranges from 3.7 to 11 per million. It can be idiopathic or secondary to autoimmune diseases, drugs, malignancy and collagen vascular diseases. Approximately 40% of TTP cases show cardiac involvement and pathologists need to be aware that it can cause sudden cardiac death. TTP needs to be differentiated from disseminated intravascular coagulopathy (DIC), Haemolytic uremic syndrome (HUS), and HELLP syndrome in pregnancy.

P47

An Analysis of the Benefits and Limitations of Post-Mortem Computed Tomography (PMCT) in Unselected Cases

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At the Sheffield Medico-legal Centre all bodies requiring a non-forensic autopsy over an 11 month period had post-mortem CT (PMCT) scans. The 752 scans, reported by consultant radiologists, allowed pathologists to consider the report alongside clinical history and external examination findings. Provision of the PMCT allowed pathologists to certify the cause of death from the clinical history, external examination findings and radiology report alone ("digital autopsy", n=334, 45%); take toxicology samples for analysis later (n=55, 7%); perform a limited invasive autopsy focussed on specific body compartments (n=331, 44%) or perform a full invasive autopsy (n=32, 4%). This facilitated workflow and targeted work onto areas of pathological interest. The three main pathologists independently produced digital:toxicology:limited:full autopsies ratios in their practice. They experienced increasing confidence in the use of PMCT alongside standard practice in this period, but were aware that the radiologists did show variable approaches to the analysis of autopsy radiology. In the invasive autopsy cases, in 58 of the limited (18%) and 12 of the full (38%) examinations, the pathologist had the same cause of death as that offered by PMCT, without additional diagnostic data benefit. In 39 of the limited (12%) and 7 of the full (22%) autopsies, the pathologist had different conclusions regarding the cause of death. Most of these cases reflected radiology overinterpreting coronary calcification, without having the ability to appreciate toxaemic pathology, soft tissue lesions, pulmonary emboli, metabolic pathology, neoplasia, airway obstruction, sepsis, bowel bleeds and microscopic cardiac disease. Whilst there are limitations to PMCT, it is a valuable tool if defined by pathologists, and may facilitate targeted examinations.

P48

Histological Type as a Predictor of Ovarian Cancer Survival in the Million Women Study

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Evidence suggests the different ovarian cancer histotypes have distinct aetiologies and risk factors. Histotype is also thought to predict survival, but few studies have sufficient cases and information to explore variation in survival by histotype with adjustment for stage at diagnosis and other potential confounding factors. Using Cox regression models, we estimated relative risks (RRs) of ovarian cancer-specific mortality, by histotype and stage, in a large prospective study of UK women, adjusted for tumour grade, age at diagnosis, and reproductive, anthropometric and lifestyle characteristics. Among 8574 women diagnosed with ovarian cancer, 4993 died from ovarian cancer during follow-up, with 42% 5-year survival and 33% 10-year survival for deaths from ovarian cancer. 3991 women had information on both histotype and stage at diagnosis. There was a strong trend of worse survival with increasing stage of disease, after adjusting for histology and other factors. Women with stage IV disease at diagnosis had a twelve-fold adjusted relative risk of dying compared to women with stage I disease (RR=11.42, 99% CI: 8.89-14.68). Survival also varied significantly by histology, after adjustment for stage of disease and all other factors. Compared to cases with high-grade serous carcinoma (the most common type), serous borderline and low-grade carcinomas, and mucinous borderline tumours, both had significantly decreased adjusted risks of dying (RR=0.44, 0.27-0.71; RR=0.11, 0.03-0.41, respectively), and endometrioid tumours had a just-significant reduction in risk (RR=0.75, 0.57-1.00), while clear cell tumours had a non-significant increased risk (RR=1.33, 0.98-1.82). Age at diagnosis had a modest effect, after adjusting for all other factors. These results confirm that stage at diagnosis is the strongest predictor of ovarian cancer survival. Tumour histotype remains a strong predictor of survival even after adjustment for stage, confirming its clinical importance.

P49

Eukaryotic Initiation Factor 4E-Binding Protein 1 (4EBP1) is an Independent Predictor of Poor Outcome in Ovarian Cancer

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Purpose of the Study: Ovarian cancer is associated with the highest mortality rate among gynaecologic malignancies. There is a need to refine classification of ovarian cancer and identify novel targets. The mammalian target of rapamycin (mTOR) pathway has a crucial role in the regulation of translation of specific proteins associated with ovarian cancer progression. The major downstream effectors of mTOR are eukaryotic initiation factor 4E-binding protein 1 (4EBP1) and ribosomal protein S6 kinase (p70S6K). We aimed to investigate the prognostic role of 4EBP1 and p70S6K in ovarian cancer.
Methods: Investigation of 4EBP1 and p70S6K protein expression, was carried out on tissue microarrays of 195 consecutive ovarian epithelial cancers treated at Nottingham University Hospitals (NUH) between 2000 and 2007. Clinicopathological and outcome data were collected.
Summary of Results: High cytoplasmic expression of both 4EBP1 and p70S6K was associated with serous type carcinoma (p=0.005 and p=0.02 respectively). High cytoplasmic expression of 4EBP1 was seen more frequently in cases treated with early debulking (p=0.005). Univariate outcome analysis showed an inverse association between 4EBP1 expression and overall survival (p=0.005) and development of local recurrence (p=0.005). P70S6K showed inverse association with local recurrence (p=0.002). Multivariate analyses indicate that independent predictors of ovarian cancer recurrence were 4EBP1 (p=0.03, HR= 1.7 and 95%CI: 1.1-2.7), histological grade and tumour stage.
Conclusions: 4EBP1 expression predicts local recurrence in ovarian cancer patients. As a translational repressor protein, it could be used as a potential biomarker for prognostic stratification and treatment decisions.

P50

An Audit of Cervical Loop Histopathology Reporting

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Background: This audit was carried out to determine the completeness of local Loop Excision of Transformation Zone (LLETZ) histology reporting in line with criteria described by the Royal College of Pathologists guideline "Tissue pathways for gynaecological pathology". The results of the first cycle were presented at the histopathology consultants meeting and the hospital proforma was amended to include crypt involvement and the presence of pathological features. Consultants were reminded to use the LLETZ reporting proforma and the audit cycle was completed in July 2017.
Method: This was a retrospective selection of all cases for a 1 year period between 01/04/15 and 31/3/16 and a 2 month period between 21/03/17 to 21/05/17. We reviewed histology reports on the laboratory information system and recorded whether key data items were included in each report.
Results: Reporting of macroscopic description and features that would impair interpretation was 100% in both cycles of the audit. Reporting of the CIN/CGIN grade was 99.6% and 100% in the first and second cycles respectively. Reporting on whether invasion was present was 93% and 100%. Reporting provisional FIGO stage (where invasive disease found) was 87.5% in the first cycle but no cases of invasive disease were found in the second cycle. Reporting excision margins was 98.5% and 100%. Reporting of crypt involvement (where CIN present) was 17% and 100%. Reporting of pathological features (e.g. inflammation or HPV related changes) was 28% and 88%.
Conclusion: The first cycle of the audit showed that implementation of an audit proforma does yield very high reporting of key criteria, but there will be minimal reporting of any features not on the proforma. Implementation of a revised proforma including all key criteria yielded near perfect reporting of key criteria. Thus proformas should be reviewed and updated regularly to ensure all relevant reporting criteria are included.

P51

Primary Gestational Fallopian Tube Choriocarcinoma Arising in a Dizygous Twin Pregnancy

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Purpose of the Study: Choriocarcinoma is an incredibly rare and malignant subtype of gestational trophoblastic disease that may coincide with normal, molar or ectopic pregnancies (1, 2). Choriocarcinoma associated with ectopic pregnancy has an incidence of 1.5/million (3). Fallopian tube choriocarcinoma is most frequently gestational, and is associated with serious morbidity, as by the time of diagnosis, metastasis is highly likely due to rupture of the tubal wall into the pelvic cavity.
Methods: We report a case of primary gestational tubal ectopic choriocarcinoma that ruptured and metastasised to the lungs, arising in a dizygous twin pregnancy, where the intrauterine foetus was successfully delivered.
Summary of Results: A 37 year-old pregnant female, who was fit and well, with no significant past medical or obstetric history, developed intermittent left iliac fossa pain at 19/40. At 34/40 weeks she presented acutely to our regional centre. Pelvic ultrasound suggested an ovarian torsion. Histology revealed choriocarcinoma of the fallopian tube (no products of conception were found), normal placenta, omental deciduosis and benign peritoneal fluid cytology (second review completed by a paediatric pathologist). CT Pulmonary Angiogram demonstrated multiple bilateral lung nodules, which following open thoracotomy and wedge-biopsy were confirmed histologically as metastatic choriocarcinoma by a specialist pulmonary pathologist. Post-operatively the patient recovered well and commenced chemotherapy at a specialist trophoblastic unit. The metastatic deposits disappeared and the patient is still alive.
Conclusions: Primary gestational fallopian tube choriocarcinoma is extremely rare, and should always be considered whilst examining ectopic pregnancy specimens. To our knowledge, this is the first report of such case occurring as part of a dizygous twin pregnancy.

P52

Lymphoglandular Bodies: A Diagnostic Pitfall

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Lymphoglandular bodies are regarded as specific for lymphoid tissue and have been reported to be helpful in distinguishing lymphomas from other small round cell tumours including embryonal rhabdomyosarcomas. We present a case of a pelvic mass in a 75 year old lady submitted for intraoperative frozen section. Touch imprint smears were performed at the same time and stained with Giemsa. Intraoperative frozen sections of the fleshy pelvic mass showed a malignant tumour containing atypical cells, the morphology of which was markedly distorted by freezing artefacts. A touch imprint performed contemporaneously showed discohesive atypical round cells with cytoplasmic fragments interpreted as lymphoglandular bodies. An intraoperative diagnosis of a high grade malignant tumour was proffered. Accurate subtyping was deferred to paraffin sections. Further sections and immunohistochemistry revealed the tumour to be a carcinosarcoma of tuboovarian origin with predominant rhabdomyosarcomatous differentiation. Abundant lymphoglandular bodies defined as more than 20 lymphoglandular bodies per high power field are considered characteristic of lymphoid malignancies. However we feel that this is not always reliable as demonstrated in our case. We also recommend the use of touch imprint smears in specimens sent for frozen sections where the cells are too fragile to withstand the cryo procedure.

P53

Genotyping of Possible Hydatidiform Moles May Lead to Incidental Findings of Abnormalities with Implications for Future Pregnancies

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Purpose of the Study: In this country suspected Gestational Trophoblastic Disease (GTD) cases are referred to a specialist centre.The pathology report,blocks and slides are submitted to a dedicated laboratory where Flow Cytometry and p57 immunohistochemistry (IHC) is undertaken.A revised report is then issued.A third of cases are confirmed non molar requiring no follow up.A subset of inconclusive cases are submitted for molecular genotyping in collaboration with our regional genetics service.Prior to genotyping, patient is registered and information sheet given with a saliva sample request.Reported here is a case of Trisomy 21 histologically mimicking a hydatidiform mole (HM) ,the diagnostic pathway and following multidisciplinary discussion, a subsequent change in practice to reflect issues of consent.
Methods: Histological review demonstrated hydropic chorionic villi with abnormal trophoblast proliferation.Flow cytometry was diploid and p57 IHC was retained excluding a diandric conception.Genotyping was undertaken in view of the abnormal morphology employing DNA extracted from laser-microdissected villi and a maternal saliva sample.
Summary of Results: Using Promega PowerPlex 16HS system comprising total of 16 microsatellite markers, the case showed biparental inheritance confirming non molar conception.However, one chromosome 21 marker indicated possible trisomy.Further analysis with Devyser Compact v3 kit containing 6 additional chromosome 21 markers confirmed evidence of trisomy.A revised report including this result was issued.
Conclusions: Aneuploid conceptions may histologically mimick HM.Genotyping of these cases lead to incidental findings of genetic abnormalities with implications on future pregnancies and hence serum screening should be considered.Inorder to include such findings in the final report,the patient information letter was changed to reflect this possibility and to provide an opportunity for the patient to refuse further confirmatory investigations.

P54

An Audit of the Lower Limit of DNA Concentration for Clonality Testing in Haematological Malignancies

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A clinical audit was performed on all Identiclone gene clonality assays carried out in NHS Lothian over a 24 month period. The results were reviewed to determine the numbers of failed samples and whether there was a correlation between failure and DNA concentration. The aim of this work was to determine the lower limit of DNA concentration acceptable for a successful assay. Of 195 samples assessed, 42 failed analysis and 18 yielded partial results. Of the 42 samples which failed analysis, 36 contained less that 2 ng/µl DNA, 2 contained less than 3 ng/µl DNA, and 4 contained more than 3 ng/µl DNA. Of the 18 samples which yielded partial results, 6 contained less than 2 ng/µl DNA, 2 contained less than 3 ng/µl DNA, and 10 contained more than 3 ng/µl DNA. Full results were obtained for 135 samples, of which 13 had less than 2 ng/µl DNA. Of these 13 samples, 8 needed repeat testing prior to reporting, 3 samples failed analysis of at least one tube, and 2 samples had a DNA ladder amplified up to 300 nucleotides suggestive of low quality samples. The Biomed II guidelines recommend a minimum of 50 ng/µl DNA for testing. However, only 19/195 samples (10%) had 50 ng/µl or more of DNA. This is possibly due to the majority of DNA samples tested in NHS Lothian being extracted from formalin fixed and paraffin embedded (FFPE) tissue samples, rather than peripheral blood. Formalin fixation is known to degrade DNA and with routine diagnostic samples often being scanty in size it is important to set a threshold for the lower limit of DNA quantity which still ensures good quality results. This audit resulted in the following recommendations and change of practice; samples with less that 2 ng/µl DNA are not tested, and samples with between 2-5 ng/µl DNA or size ladder amplification below 300 nucleotides are reported with a cautionary proviso.

P55

Sentinel Lymph Node Biopsy for Oral Carcinoma: Is the Current UK Laboratory Protocol Necessary?

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Purpose of the Study: Recent UK recommendations for management of clinically N0 necks in early stage oral carcinoma include the option of sentinel lymph node biopsy (SLNB) with a labour intensive three-stage laboratory protocol. Our study examined: the necessity for such thorough laboratory methods, the reproducibility of microscopic interpretation and our alignment to national report turnaround time guidelines.
Methods: 47 consecutive SLNBs previously reported as positive from 36 patients with cT1/2N0 oral squamous cell carcinoma were examined. Slides were re-examined and the number of positive SLNs, the protocol stage at positivity, the first cut level positive (protocol stage I, II or III) and the number of positive levels (stage II or III) were recorded. Diagnostic disagreement was resolved by a third specialist pathologist and percentage agreement calculated for inter-observer variation. Report turnaround times were measured against the current standard of 80% within 10 calendar days.
Summary of Results: 24 of the 47 SLNBs were positive at stage I (single index H&E). At stage II or III, 17 nodes were positive on a step single section; 3 were positive on 2 step sections; 4 were positive on 4 step sections and 2 were positive on 5 step sections. Inter-observer variation showed 83% diagnostic agreement at initial review. 63% of specimens were reported within 10 days (mode=7 days). 27 (57%) of the SLNBs were first cut level positive.
Conclusions: Our data indicate that current laboratory protocols for SLNB in oral carcinoma are necessary; avoidance of immunohistochemistry on serial step sections will fail to identify 40% of patients with occult nodal metastases.

P56

Recognising differentiated dysplasia in the head and neck - morphological parameters and correlation with cytokeratin 13 and cytokeratin 17 staining

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Differentiated dysplasia in the head and neck is not acknowledged by the WHO, despite recent reports associating it with almost 80% of squamous cell carcinoma (SCC) of the oral cavity. As this lesion has a subtle appearance, and can progress to SCC without exhibiting the (near) full-thickness atypia of classical high grade dysplasia, its histopathological diagnosis and grading is difficult. Combined immunohistochemistry with cytokeratin 13 (CK13) and cytokeratin 17 (CK17) is considered useful for (high-grade) dysplasia, where CK13 loss and CK17 expression can be seen. We investigated differentiated dysplasia associated with head and neck SCC (HNSCC) and explored the possibility of histological grading. We also studied the role of combined CK13 and CK17 immunohistochemistry in its diagnosis.
Dysplasia adjacent to HNSCC (2016-2017) from oral cavity and larynx were studied. The morphological features of differentiated dysplasia were enumerated, and a grading system drawn up by scoring each feature. Combined immunohistochemistry with CK13 and CK17 was conducted, and expression patterns were correlated with the histological grading.
Differentiated dysplasia was found in 74% (62/84) of HNSCC cases. Loss of polarity and individual cell keratinisation were noted in the lower grades and increased cellularity, deep keratosis, architectural changes (cobblestoning) in higher histological grades of differentiated dysplasia. Progressive CK13 loss (median: 40% in grade 1 to 95% in grade 3) and CK17 expression (median: 5% in grade 1 to 85% in grade 3) with increasing grade was noted.
Through these preliminary results, the range of histological appearance of differentiated dysplasia was delineated. Histological grade correlated with the extent of CK13 loss and CK17 expression. Awareness of this lesion in the head and neck is crucial. These findings may guide pathologists in identifying this lesion. Study on more cases and validation with clinical data and follow up is being conducted.

P57

Raman Spectroscopy for Intraoperative Assessment of the Resection Margins in Head and Neck Surgical Oncology

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To establish the usefulness of Raman spectroscopy for intraoperative assessment of soft and bone tissue resection margins during oral cavity squamous cell carcinoma (OCSCC) surgery.
Raman spectroscopy is an non-destructive objective technique that provides (real-time) information about the molecular composition of tissues. It can be used ex-vivo and in-vivo without tissue preparation.
Raman ex-vivo experiments were performed on freshly resected OCSCC specimens. The studies have shown that Raman spectroscopy can discriminate OCSCC from the surrounding healthy soft tissue, with 99% sensitivity and 92% specificity (170 point measurements/ 14 patients), and can be used to determine the OCSCC border (25 mapping experiments/ 20 patients). Furthermore, a recent study have shown that Raman spectroscopy can detect OCSCC in bone resection surfaces with high sensitivity (96%) and specificity (83%) during mandible resections (26 mapping experiments/ 22 patients).
Our results are promising and show that an objective technique like Raman spectroscopy could be applied intraoperatively to evaluate resection margins (of soft and hard tissue specimens). Therefore this technique can solve the lack of intraoperative assessment for hard tissue and allow the assessment of complete soft tissue resection margins.This method could improve the currently reported pure numbers of adequate resection margins, varying between 2% and 85%.

P58

High Endothelin-Converting Enzyme-1 Expression Independently Predicts Poor Survival of Patients With Oesophageal Squamous Cell Carcinoma

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Purpose of the Study: Patients with oesophageal squamous cell carcinoma (OSCC) have poor survival and high recurrence rate, thus an effective prognostic biomarker is needed. Endothelin converting enzyme-1 (ECE-1) is responsible for biosynthesis of endothelin-1, which promotes growth and invasion of human cancers. The role of ECE-1 in OSCC is still unknown.
Methods: We enrolled patients with OSCC who provided pre-treated tumour tissues. Tumour ECE-1 expression was evaluated by immunohistochemistry and was defined as either low or high expression. Then we evaluated if tumour ECE-1 expression had any association with clinicopathological findings or predicted survival of patients with OSCC.
Summary of Results: 54 of 99 patients with OSCC had high tumour ECE-1 expression, which were significantly associated with more advanced clinical stages (p=0.07) and lymph node metastasis (p=0.04). In addition, tumour ECE-1 expression independently predicted survival of patients with OSCC and the 5-year survival was poorer in patients with high tumour ECE-1 expression (p=0.016). Among patients with locally advanced and potentially resectable OSCC (stage II and III), 5-year survival was poorer with high tumour ECE-1 expression (p=0.003). High tumour ECE-1 expression also significantly predicted poorer survival of patients in this population.
Conclusions: In patients with OSCC, high tumour ECE-1 expression might indicate high tumour invasive property. Therefore, tumour ECE-1 expression could be a good biomarker to identify patients with worse survival and higher risks of recurrence, who might benefit from the treatment by ECE-1 inhibitor.

P59

Sclerosing Polycystic Adenosis of the Parotid Gland: An Interesting Case Report of a Rare Entity

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Sclerosing Polycystic Adenosis (SPA) of the parotid gland is a recently described, rare entity, with only 45 cases reported within the literature. The pre-operative assessment of these lesions is challenging, and usually the surgical excision specimen is required for accurate diagnosis.
We present a case of an 81 year old female who developed a slow growing, 1.5 cm, lump in the left parotid. Clinically this was felt to be benign. On initial Fine Needle Aspiration (FNA) the cytological features of polygonal cells with granular cytoplasm lead to a diagnosis of an "oncocytic neoplasm with features of acinic cell carcinoma". On the excision specimen the macroscopic appearances of the lesion were those of a firm, fibrous, well defined lump within the parotid gland. Microscopically the lesion showed the typical features described for SPA; it was well circumscribed and composed of a fibrous, hyalinised stroma containing dilated glands and scattered acinar and cribriform structures. Clear cells, vacuolated cells and onococytic-like cells were all present within the lesion and importantly some cells contained DPAS positive, coarse, eosinophilic granules, which are the hallmark features of SPA. In our case there was no evidence of dysplasia or malignancy, which has been described in other case reports and has comparable appearances to ductal carcinoma in situ (DCIS) seen within the breast.
Our case confirms the difficulties and challenges of a FNA cytological diagnosis for this entity, which can lead to an over diagnosis of malignancy. Lastly, we will discuss the emerging evidence that this lesion is a clonal proliferation.

P60

Acinic Cell Carcinoma With High-Grade Transformation

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A 78 year old female presented with a left sided partial Bell's palsy and a mass in level II left neck as well as a bulky parotid. Cytology of the parotid and neck showed features of a high grade malignant tumour likely from salivary gland origin. A total paroidectomy specimen contained predominantly high grade tumour composed of infiltrative pleomorphic cells with mitosis and necrosis. In addition, small foci of neoplastic cells with granular cytoplasm arranged in acinar and microcystic architecture were seen. The neoplastic cells were positive for DOG1, CK5 and negative for p63, S100, p16, AR and EBV. These features are in keeping with an acinic cell carcinoma with high grade transformation. This case is of interest as acinic cell carcinoma with high-grade transformation is a rare variant of acinic cell carcinoma, composed of both a conventional low-grade and a separate high-grade component. It highlights the importance of extensive sampling to ensure the appropriate diagnosis is achieved.

P61

Diagnostic Difficulty of Pleomorphic Adenoma Versus Adenoid Cystic Carcinoma

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A 70 year old female presented with increasing pain from a tumour of the right accessory lobe of parotid, which had been present for 10 years. Cytology of the lesion was reported to be a pleomorphic adenoma with adenoid cystic features. However, on resection of the specimen an adenoid cystic carcinoma was found with no evidence of pleomorphic adenoma. This case illustrates the common and difficult differential diagnosis between pleomorphic adenoma and adenoid cystic carcinoma. Pleomorphic adenoma can show the cribriform architectural characteristics of adenoid cystic carcinoma making the cytological diagnosis on FNA sample difficult. With limited material, it may not be possible to make a confident distinction between these tumours. However, majority of adenoid cystic carcinomas carry a fusion gene called MYB-NFIB. In this case, we suggest that immunohistochemical expression and/or chromosomal translocation can be used as a diagnostic aid for the pre-operative diagnosis of pleomorphic adenoma versus adenoid cystic carcinoma.

P62

A Potential Pitfall in Diagnosing Primary Sinonasal Melanoma

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Introduction: Primary sinonasal melanoma is rare. Accurate diagnosis is vital because it informs primary surgical excision, however wide variation in morphology and immunophenotype can make diagnosis challenging. Usual histological features are often absent, so diagnosis depends on a high degree of suspicion and a complete immunophenotype.
Case Report: A 64 year old male was incidentally noted to have a right maxillary tumour on imaging performed for an acute cerebrovascular accident
Radiological Findings: CT and MRI Head showed a right maxillary sinus tumour, filling the sinus and indenting the dura at the roof without direct connection with intracerebral tissue.
Histological Findings: Initial biopsy showed an infiltrative subepithelial neoplasm composed of nests of small round blue cells that were largely plasmacytoid with vesicular nuclei and dense eosinophilic cytoplasm. There was focal rhabdoid morphology. No junctional activity, melanin pigment or neuroendocrine features were identified. Immunohistochemistry for synaptophysin, chromogranin, CAM5.2, CK7, CD138, Desmin,myogenin, TTF1, CD99, p63, LCA, S100, CD2, CD3, CD4, CD5, CD7, CD8 and ALK1 was negative. CD56 was positive in the initial panel. The broad differential diagnosis included rhabdomyosarcoma, sinonasal SMARCB1 deficient sinonasal carcinoma, neuroblastoma and lymphoma, however subsequent MelanA and HMB-45 diffuse positivity confirmed melanoma.
Management/Conclusion: Melanoma is a great mimicker and can be S100 negative and CD56 positive. CD56 positivity in melanoma has been reported as a poor prognostic indicator. Three melanoma markers should be applied before the diagnosis is out-ruled, especially in sinonasal tumours. Treatment is primarily surgical and the patient underwent primary resection as opposed to chemoradiotherapy that would be appropriate for other tumours in the initial differential.

P63

An Unusual Case of Metastatic Hepatocellular Carcinoma to the Mandible: A Case Report and Review of the Literature

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The mandible is an uncommon but recognised site for metastasis from distant tumours. In men, lung primaries are the most common type of jaw metastasis and in women, breast adenocarcinoma. It is extremely rare for hepatocellular carcinoma to metastasise to the oral cavity, with less than 20 reported cases in the literature. We present an instance of such a case. A 76 year old man presented with an expansile mass associated with the left mandible, with local bone destruction. On imaging it was felt to be suspicious of a giant cell lesion. A biopsy revealed a papillary oncocytic tumour with moderate nuclear atypia, frequent mitsoses and foci of lymphovascular invasion. A provisional diagnosis of a salivary gland primary tumour was considered. Initial immunohistochemistry showed positivity for AE1/3, CK20, and CEA. Tumour cells were negative for CD10, PSA, PAP, TTF1 and CDX2. Subsequent staining for HEPAR-1 showed patchy positivity and a Fouchet's Van Gieson showed focal bile. A diagnosis of metastatic hepatocellular carcinoma was made and a subsequent CT-angiogram showed multiple liver lesions. The mandible is an extremely rare site for metastasis of hepatocellular carcinoma and it is unusual for it to present in this way. This case highlights the importance of immunohistochemistry in making the diagnosis and raises awareness of this unusual diagnosis at this site.

P64

Follicular Lymphoma within a Warthin's Tumour: A Case Report and Review of the Literature

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Warthin's tumour is a relatively common benign parotid tumour with a characteristic lymphoid stroma and bilayered oncocytic epithelium. Very rarely, this ordinarily benign lymphoid stroma can be found to contain a lymphoma. There are approximately 12 reported cases in the literature of this concurrent pathology. We present a case of a 78 year old female with a previous medical history of both follicular lymphoma and diffuse large B cell lymphoma, who presented with a right cervical lymphadenopathy in the level II region. A core biopsy showed a tumour comprising a lymphoid stroma and bilayered oncocytic epithelium. Normal lymphoid architecture within the lymphoid component was effaced and replaced with a small round blue cell tumour comprising tightly packed small cleaved cells and larger cells with more prominent nucleoli. Tumour cells were positive for BCL-2, BCL-6, CD10, CD20, CD79 and were negative for CD23, CD5, cyclin-D1, LMP-1 and IRF4. Ki67 showed a moderate proliferation index. A local diagnosis was made of grade 2 follicular lymphoma with an abnormal germinal centre B cell phenotype, colonising the lymphoid stroma of a Warthin's tumour. While rare, this diagnosis represents a recognised phenomenon and this interesting case illustrates the importance of thorough histological examination of the lymphoid stroma within Warthin's tumours.

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Oncocytes in Polymorphous Salivary Adenocarcinoma: Histological and Immunohistochemical Observations

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Purpose of the Study: Although oncocytes are established in epithelial salivary tumours other than oncocytoma or Warthin tumour, very little attention has been paid to the occurrence of such cells in polymorphous (previously known as polymorphous low-grade) adenocarcinoma. The present study pursues this and explores sub-cellular events of possible pathogenetic significance.
Methods: A total of 14 cases of polymorphous salivary adenocarcinoma were histologically diagnosed by this author over a six-year period (4 males and 10 females; ages ranged from 38 to 82 years with a mean of 65.7 years; 13 were located in minor salivary glands and 1 in accessory parotid). Several paraffin-embedded blocks of every case were extant. Haematoxylin and eosin stained sections obtained from them, were light microscopically studied for presence of oncocytes. Selected cases were further examined by means of immunohistochemical techniques that have been found of value in assessing mitochondria (TOMM20), metabolic pathways (monocarboxylate transporter 1 and 4; MCT1 and MCT4) and lysosomal activities (CD63).
Summary of Results: A single case (male, 76 years, palate; 7%) showed 'pink' oncocytes within the tumour parenchyma, which were in small, solid or inchoate tubular arrangements. In comparison with conventional tumour cells, the oncocytes were intensely TOMM20 (+), and showed plasmalemmal MCT1 and decreased CD63 immuno-staining; they were not detected in the MCT4 preparations or adjacent minor salivary gland. In another case (female, 70 years, palate), oncocytic ducts present in the adjacent gland had been trapped within the tumour parenchyma.
Conclusions: Oncocytes in polymorphous salivary adenocarcinoma appear uncommon and unrelated to their presence in adjacent glands. Autophagy is likely decreased in these cells, which may influence their mitochondrial load. The oncocytes are able to take up lactate usable for respiratory fuelling – hence, their mitochondria may not be dysfunctional.

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Histology and Immunohistochemistry of a Familial Salivary Adenocarcinoma with Endocrine-Like Features

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Purpose of the Study: A family with an unusual carcinoma of the salivary glands has been reported (Michaels L et al. Am J Med Genet 1999; 83: 183-186). The present study aims at refining the related pathology.
Methods: Previously unreported sublingual (floor of mouth) primaries, recurrences and cervical metastases from two female members of the affected family (II.6 and III.4 of the pedigree) were examined by histology, and immunohistochemical and histochemical techniques valuable in characterising cell phenotypes/events in salivary neoplasia and cell cycle antigens.
Summary of Results: All tumours were solid, cellular and asymmetrically lobulated with a usually pushing growth pattern. They showed small eosinophilic luminal structures, often with mucous cells, which were surrounded by multiple layers of pale non-luminal cells; the appearances variously simulated atypical carcinoid and epithelial-myoepithelial carcinoma. Foci of transition between primary tumour and normal salivary glands were seen. The luminal structures showed argentaffinity attributable to lipofuscin and regularly expressed a wide range of cytokeratins (CKs), including 5/6, 7, 14, and 19. The mucous cells produced neutral and acidic glycoproteins. Sporadic expression of CKs 7, 3/12 and 20 was a feature of the non-luminal cells that were also CD56 (+, plasmalemmal), PGP9.5 (+), neuron specific enolase (+), synaptophysin (-) and chromogranin (-); and did not stain for smooth muscle actin or caldesmon. Occasional argyrophilic cells were detected. S-100 protein and epithelial membrane antigen were more often expressed in the luminal structures. Staining for Ki67, p16, p21 or p53 was not seen.
Conclusions: These unique low-grade, though persistent, tumours are probably deriving from glandular parenchyma and show biphasic structural organisation. In contrast with conventional salivary neoplasia, however, their non-luminal cells show neuroendocrine-like rather than myoepithelial differentiation.

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Sarcoid Presenting in the Larynx

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Introduction: Laryngeal involvement is a rare and potentially life-threatening manifestation of sarcoidosis, estimated to affect 0.5–8.3% of patients. Clinical manifestation ranges from asymptomatic to severe, usually with airway obstruction. Infrequently the larynx is affected in isolation and the clinical suspicion is low, making diagnosis more problematic. We present the unusual scenario of sarcoid being diagnosed in a patient after laryngeal biopsy.
Case Report: A 31 year old female presented with stridor, increased respiratory rate and inspiratory wheeze, with reduced air entry in the left lower hemithorax and right base. She had a history of recurrent supraglottitis, with intensive care admission and intubation. The chest x-ray showed normal cardio-mediastinal contours, with clear lung and pleural spaces. Microlaryngoscopy demonstrated oedematous arytenoids, aryepiglottic and supraglottic folds. A biopsy of the right arytenoid was taken.
Histological Findings: Histological examination demonstrated respiratory mucosa with aggregates of lymphocytes surrounding non-necrotising epithelioid granulomas. There was no evidence of neoplasia. A Ziehl-Neelsen special stain was negative for acid fast bacilli. The findings together with the clinical picture were consistent with sarcoid.
Conclusion: Sarcoidosis is a granulomatous disease of uncertain aetiology that is difficult to diagnose and to treat. It is often a diagnosis of exclusion and the differential is wide, including neoplastic disease. The condition is underdiagnosed in the larynx because it occurs infrequently at that site and the biopsies are usually small. The diagnosis is even more difficult when there are few systemic symptoms and the clinical index of suspicion is low. This can present a great clinical challenge if there is also the need to consider prompt and invasive airway management. This case highlights the importance of the biopsy in diagnosing laryngeal sarcoidosis and thus influencing clinical management.

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Audit of pT1 Colorectal Carcinoma Detected in Bowel Cancer Screening Patients (BCSP) and Non-Screening Symptomatic Patients

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Introduction: Early pT1 colorectal carcinoma is defined by invasion of tumour into the submucosa and not beyond. Depth of invasion, histological grade, presence of lymphovascular invasion, budding, poorly differentiated clusters and resection margins are important predictors of lymph node metastasis and tumour behaviour.
Materials and Methods: This is a retrospective analysis of pT1 colorectal cancers diagnosed in the histopathology department over a period of 7 years (2010-2016). The available clinical records and histopathology slides were reviewed for various parameters.
Results: The study included 28 BCSP cases and 51 non-screening cases that had either polypectomies or colorectal resections. Male predominance with an age range of 55-74yrs was noted in BCSP cases; however, in non-screening patients the age range was 49-91 years. In BCSP cases all polyp cancers were on left side with sigmoid colon (18/28) being the commonest site. In non-screening cases, cancers were noted in both right and left sides of colon with rectum being the commonest site (22/51). The cancers were smaller in BCSP cases (width <5mm in 25/28) whereas a proportion were larger (width >5mm in 22/51) in non-screening cases. Poorly differentiated clusters were more commonly seen in non-screening cases (13/51) as compared to BCSP cases (1/28). In patients who underwent major resections, lymph node metastasis was seen in 5 non-screening cases and only 1 BCSP case.
Conclusion: BCSP cases tend to have smaller polyp cancers, all on the left side and with very low incidence of lymph node metastasis as compared to non-screening pT1 cancers.

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Malignant Bone Tumours with *H3F3A* Mutations: A Genomic and Epigenetic Study

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Introduction: Giant cell tumour of bone (GCTB) is an uncommon neoplasm of osteoblastic lineage. Approximately 98% of 'conventional' GCTB harbour missense mutations in the histone H3.3 gene (*H3F3A*), as do ~5% of histologically malignant tumours showing osteoblastic differentiation. Our aim was to characterise the genomic and epigenomic landscape of *H3F3A*-mutated malignant bone tumours with the aim of identifying molecular prognostic features.
Methods: The following investigations were performed on histologically 'conventional' (n=38) and 'malignant' (n=11) GCTB: whole genome sequencing (WGS), DNA methylation profiling, and/or SNP array. Data were compared with those generated from 'conventional' osteosarcomas without *H3F3A* mutations (n=35).
Results: Bone tumours with *H3F3A* mutations showed distinct global DNA methylation profiles, but benign and malignant tumours could not be reliably separated on the basis of methylation. WGS data suggested two distinct subgroups of *H3F3A*-mutated malignant tumour. 6/9 genomes from these tumours showed a high number of structural variants, and other changes previously reported in osteosarcomas (Group 2). The remaining 3/9 cases showed virtually no alterations (Group 1). All samples in Group 2 showed abnormalities of ploidy and/or somatic copy number alterations. Group status correlated with clinical outcome in the form of survival.
Conclusions: *H3F3A*-mutated bone tumours can be identified by their methylation profile and distinguished from all other bone tumour subtypes. Using death as an outcome measurement for *H3F3A*-mutated tumour, two distinct groups were identified on the basis of copy number and/or ploidy abnormalities: we propose that these criteria may be used to classify 'conventional' and 'malignant' GCTB, and only GCTB with 'osteosarcoma-type' molecular profiles stratified for standard of care osteosarcoma treatment. This study would benefit from a validation cohort.
Supported by a grant from the Pathological Society.

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Epigenetic Compound Screening Reveals New Therapeutic Targets for Chordoma

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Chordoma is a rare cancer, with an incidence of 1 in 800,000 of the population, showing notochordal differentiation and with a median survival of 7 years. We have previously demonstrated that EGFR inhibitors represent almost uniquely the family of compounds to exert an effect on chordoma cell lines proliferation, however not all cell lines respond and drug resistance is likely to occur. Genomic studies have revealed that chordomas do not harbour recurrent alterations in kinases whereas chromatin-remodelling genes are altered in at least 20% of cases. The transcription factor brachyury (T), the diagnostic hallmark of chordoma, is strongly implicated in its pathogenesis and is regulated during embryonic development at the epigenetic level, suggesting that epigenetic inhibitors may represent a therapeutic approach for this disease. In this study we have undertaken a medium throughput focused compound screen using validated small molecule inhibitors of enzymes involved in chromatin biology (n=91) targeting readers, writers and erasers of the "chromatin code". Compounds were assessed for their ability to decrease cell viability in an Alamar blue assay in five chordoma cell lines (UCH1, UCH2, MUG-Chor, UM-Chor, UCH7). Screening revealed activity in a range of compounds targeting the jumonji domain-containing lysine demethylases including GSK-J4 and KDOBA67, two structurally closely related compounds that mainly target KDM6A (also known as UTX) and KMD6B (JMJD3). The compounds were effective in all cell lines tested and, in contrast to EGFR inhibitors, promoted a strong downregulation of brachyury at the transcriptional and protein level. Chordoma cell lines were also sensitive to halofuginone, a highly specific inhibitor of the enzyme glutamyl-prolyl tRNA synthetase that has already been assessed in phase I autoimmunity clinical trials. In conclusion, we have identified epigenetic and metabolic pathways that represent potential novel targets for the treatment of chordoma.

P71

Effects of Glycation on Degradation of Collagen: An *In Vitro* Investigation

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Purpose of the Study: Although non-enzymic glycation of longstanding proteins is ubiquitous and increases with age, its effects on the degradation of collagen, a feature of tissue remodelling, infection and invasion, has received little attention. The present investigation attempts to remedy this via development of an in vitro model.
Methods: Native type 1 skin and tendon collagen, and gelatin derived from denatured type 1 porcine skin, were in vitro glycated via exposure to glucose in solution for 28 days. Effectiveness and extent of glycation were measured by assaying for hydroxymethylfurfural (HMF). Type 1 collagen was thus found to be modified by up to 30 nmol HMF formed per mg protein compared to non-glycated controls; exposure of gelatin to 0.125–1.25M glucose concentrations resulted in a dose-dependent increase in glycation up to 60 nmol HMF per mg protein. The glycated proteins were tested for susceptibility towards mammalian trypsin, an extracellular protease fraction of Enterococcus faecalis OG1RF, and Clostridium histolyticum collagenase, by a dot-blot assay after Sirius Red F3B staining (for type 1 collagen) and by a radial gel diffusion assay after staining with bromocresol green (for gelatin).
Summary of Results: Gelatin degradation by trypsin was reduced with increased glycation. In contrast, degradation of glycated type 1 collagen and gelatin by the E. faecalis protease fraction and Clostridium collagenase was increased compared to controls.
Conclusions: The results suggest that glycated collagen peptides are rendered less capable of being degraded by host proteases, but susceptibility to bacterial proteases is increased.

P72

Optimisation of a Rapid Bone Marrow Decalcification Protocol that Preserves DNA for Subsequent Molecular Analysis

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Bone marrows that are formalin fixed and processed to paraffin require decalcification. Various acids are used for decalcification, including formic acid, hydrochloric acid, nitric acid and acetic acid, but an increasingly popular alternative for bone marrow trephines is EDTA. It is recognised that, in order to perform subsequent molecular analysis, including PCR-based and sequence-based assays, the pH needs to be kept close to or slightly above 7.0, during the decalcification process, making EDTA a particularly suitable solution. Various institutions have used 5–10% (w/v) EDTA, pH 7.0–7.4, and, in our experience, this requires 48 hours' decalcification for bone marrow trephines, after an initial 24 hour fixation period. To improve turnaround time, we tested a decalcification protocol using 15% EDTA, pH 7.2, for 24 hours on 3 trephine-sized pieces of femoral head. We demonstrated that this protocol does not alter morphology or results of immunostaining or special stains. We also demonstrated its compatibility with chromogenic in situ hybridisation for light chain RNA, fluorescent in situ hybridisation for structural chromosome rearrangements and with PCR-based clonality assays. Since this protocol gives an identical turnaround time to previous protocols that used DNA-damaging formic acid, we envisage widespread uptake of our approach in the genomic era.

P73

When You See Stripes, Think Mountant Not Zebra: A Digital Pathology Critical Incident Case Report

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Background: Digital pathology (DP) is used for teaching, consultation practice, research and primary reporting. Our institution has over three years' experience of using DP for intra-operative frozen section reporting between two separate sites. There is a limited evidence base of the problems that can be encountered when using DP in a clinical setting. We report a critical incident that led to the interruption of our digital pathology workflow.
Case Details: A frozen section slide from an active thoracic case was scanned and the displayed image comprised vertical pink stripy lines partially obscuring the morphology. A rescan was performed as per standard procedures for scanner failure. This produced an error message and the scanner was unable to focus or produce an image. One slide from the case had already been successfully scanned and this was sufficient to provide enough useful clinical information. This incident was therefore classified as a 'near miss'. The overall consequence of this incident was the redeployment of a pathologist to report frozen sections from glass slides and subsequent disruption to departmental workflow. An incident evaluation showed that coverslip mountant had inadvertently smeared onto the objective lens. This created the characteristic stripy lines on the digital image, and subsequent scanner failure. The problem was resolved by an engineer visit, requiring clearning hardened mountant from the lens with xylene, within 48 hours of the failure. BMS Staff have now been trained to do this themselves if required.
Discussion: We have reported a novel finding (the 'zebra' sign) that can indicate serious problems in a digital pathology workflow. This case highlights the importance of consistently high quality slide preparation prior to digital scanning. Moreover standard operating and failsafe procedures are shown to play a critical role in the modern digital laboratory.

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An 'Interactive Human Body': A Pathology Public Engagement Tool

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Purpose of the Study: It is widely accepted that public engagement in medicine has far reaching benefits. This is recognised by the Royal College of Pathologists and has resulted in their Public Engagement Programme. Currently, there is no freely accessible tool that would enable members of the public to navigate through different body systems, and simulate the work of the pathologist. A tool such as this would help foster an increased understanding of pathology and the role of the pathologist. We aimed to create an 'Interactive Human Body' designed specifically for this purpose.
Methods: We designed the 'Interactive Human Body' as an interactive HTML5 web system, using a stacked 2D layout of vibrantly coloured transparent organs to avoid over complication and hotspot occlusion, frequently found in 3D variants. The tool was primarily designed to be accessed from a large touch-screen display, as well as standard desktop and mobile devices. The design was arranged by organ system, including brain, lung, breast, stomach, liver, bladder, colon and skin. On selecting an organ, corresponding basic information and a diagram appear. The user can then view a 'normal' whole slide image (WSI) of that organ and compare this with a WSI showing a common abnormality. Features of interest within the WSIs have been annotated using lay terminology.
Summary of Results: The finalised 'Interactive Human Body' can be viewed at ***http://www.virtualpathology.leeds.ac.uk/public/body/***. Images of the tool will be shown to demonstrate its functionality. The tool has been used at a number public engagement opportunities at the University of Leeds with resounding interest and enthusiasm from members of the public.
Conclusions:
We have created a freely accessible tool that allows users to simulate the work of the pathologist. We invite your attendees to refer members of the public to this tool in order to increase understanding and improve public engagement in pathology.

P75

Validation of the Oncomine™ Focus Panel for Next Generation Sequencing of Clinical Tumour Samples

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Purpose: The clinical utility of Next Generation Sequencing (NGS) for a diverse range of targets is expanding, increasing the need for multiplexed analysis of both DNA and RNA. However, translation into daily use requires a rigorous and comprehensive validation strategy. The aim of this clinical validation was to assess the performance of the Ion Torrent Personal Genome Machine (IonPGM™) and validate the Oncomine™ Focus DNA and RNA Fusion panels for clinical application in solid tumour testing of formalin fixed paraffin embedded (FFPE) tissue.
Methods: Using a mixture of routine FFPE and reference material across a variety of tissue and specimen types we sequenced 86 and 31 samples on the Oncomine™ Focus DNA and RNA Fusions assays respectively. This validation considered a number of validation parameters including sequencing performance, limits of detection (LOD), analytical sensitivity and specificity, and repeatability as well as the clinical robustness of the bioinformatics pipeline for variant detection and interpretation.
Summary of Results: The Oncomine™ Focus DNA assay had a gene and variant based sensitivity of 99.1% and 97.1% respectively and an assay specificity of 100%. The Oncomine™ Focus Fusion panel has an assay sensitivity and specificity of 100%. We observed variable inter and intra gene LODs which ranged from 5.3-11% for 19/25 genes assessed. Melanoma, NSCLC and GIST samples showed a good performance in NGS, whilst colorectal samples experienced the highest target amplicon dropout at our specified coverage. In addition to assay validation we have performed a validation of bioinformatic pipelines and suggest the use of multiple analysis software to ensure identification of clinically applicable variants.
Conclusion: With an increasing number of clinically actionable targets requiring a variety of methodologies, NGS provides a cost effective and time saving methodology to assess multiple targets across different modalities.

ABSTRACT REVIEWERS

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