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Supplement Contents

KEY

Presenter = Ⓟ

Abstracts marked ◆ have not been subjected to peer review

COVER PHOTOGRAPHS
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The following Invited Speaker abstracts marked ◆ have not been subjected to peer review

◆ Exploring the Junk in Our Genomes: The Blurred Boundary Between Coding and Non-Coding RNAs

K Douka[1]; D Wang[2]; I Birds[1]; S Clayton[3]; A Byford[3]; J Deuchars[3]; A Whitehouse[1]; Ⓟ JL Aspden[1]

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Only ~4% of the human genome encodes protein, whilst ~85% is transcribed into RNA. These transcribed regions give rise to non-coding RNAs, many of which are >200nt in length, and termed long non-coding RNAs (lncRNAs). Mutations in protein-coding genes have been thoroughly mapped and their contribution to human disease dissected. Yet we have limited understanding of how mutations in the other parts of the genome impact human health. It is therefore important to understand lncRNA functions and their potential contribution to human disease. LncRNAs exhibit more tissue and developmental-stage specific expression than mRNAs. They are enriched in the nervous system and testes. Several lncRNAs regulate neuronal differentiation and lncRNA mis-regulation has been implicated in neurological disorders (e.g. BACE-AS1 in Alzheimer's Disease). The molecular function of lncRNAs is highly dependent on their nucleotide sequence and the proteins they interact with, in a sequence specific manner. In fact, many lncRNAs have been shown to interact with ribosomes and a small proportion show evidence of translation, resulting in the synthesis of small peptides. However, these translation events remain controversial and their medical importance poorly understood. To determine the biological importance of neuronal lncRNA-ribosome interactions, we have characterized lncRNAs during neuronal differentiation (SH5Y5Y cells). qRT-PCR of translation complexes indicates that many neuronal lncRNAs are associated with ribosomes. To globally detect lncRNA-translation machinery interactions we have performed Poly-Ribo-Seq. We have found ~180 lncRNAs upregulated during differentiation, ~70% of which are associated with ribosomal complexes e.g. LINC01116. ~150 lncRNAs show evidence of translation. Many of these lncRNA translation events are regulated during neuronal differentiation. We are currently investigating the potential function of these lncRNAs-ribosome interactions, in neuronal differentiation.

◆ How will the 100,000 Genomes Project Affect Histopathologists?

Ⓟ JL Jones

Barts Cancer Institute, QMUL, London, UK

Pathology and Pathologists have been central to the delivery of the 100,000 Genomes Project, and will continue to be key in translating the promise of Genomic Medicine into patient benefit. Appropriate tissue sampling is the cornerstone of diagnostic pathology and is of the same importance to all genomic testing: tumour content, in-situ versus invasive elements, mixed tumour morphology – all might impact on the interpretation of a test performed on homogenized samples. The handling of tissues and its optimal fixation also underpins accurate diagnosis, but the deleterious effect of formalin fixation on whole genome sequencing (WGS) required the implementation of fresh tissue pathways for which the logistical challenges are not inconsiderable. A number of laboratories have employed innovative solutions, such as vacuum packing, but this is not without its own challenges. As WGS results are returned from the 100,000 Genomes Project, Pathologists are joining Genomic Tumour Advisory Boards, to integrate these genomic results with the histopathology – for many there is a knowledge gap and the profession needs to be proactive in filling this. The legacy of the 100,000 Genomes Project is the NHSE Genomic Medicine Service. Again, Pathology is at the core of this, being primarily responsible for requesting appropriate tests and providing appropriate samples. The service is in its infancy and Pathology has a role in shaping it, which we should embrace. Genomic analysis will not replace histopathology, it will enhance it – in diagnostic, prognostic and therapeutic evidence. We are all Molecular Pathologists now.

◆ Latest Developments in Lynch Syndrome and the Reproductive Tract

Ⓟ IM Frayling

Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK

Remarkably, from the Prospective Lynch Syndrome Database (www.PLSD.eu) we now understand that Lynch syndrome (LS) is a sex-limited condition characterised by a predisposition to endometrial cancer in females who have pathogenic variants affecting one of the four DNA mismatch repair (MMR) protein genes: *MLH1, MSH2, MSH6* and *PMS2*. Additional predisposition to ovarian cancer is seen in females, as well as to colorectal cancer in both males and females, who harbour such variants affecting *MLH1, MSH2* and *MSH6*. We also realise from the PLSD that survival of both endometrial and ovarian cancer in LS is good compared to the general population. In addition, ovarian cancer survival in LS is especially good compared to those predisposed by pathogenic variants in *BRCA1/2*, because the biology is different: ovarian LS tumours are generally of non-serous type. Compared to large bowel tumours, we understand rather less about how and why gynaecological cancers arise and develop in LS, but parallels are emerging in the critical importance of the immune system and non-dysplastic premalignant changes. The reasons why different propensities to cancers are seen with the different genes has up to now been obscure, but some clues are perhaps becoming apparent. LS is *not* a rare disease, and this increased understanding is important in improving the diagnosis and care of all cancer patients, not just those with LS.

◆ Molecular Pathology of Diagnostic Evaluation in Lynch Syndrome

Ⓟ MJ Arends

University of Edinburgh, Edinburgh, UK

Lynch Syndrome (LS) is an inherited autosomal dominant disorder predisposing individuals to a range of cancers, most commonly endometrial and ovarian cancer in the female reproductive tract, and colorectal cancer in the gastrointestinal tract. It is caused by pathogenic mutations of the DNA mismatch repair (MMR) pathway genes, MLH1, MSH2, MSH6 and PMS2, which prevent the detection and repair of DNA replication errors, mismatches and insertion/deletion loops, and some other nucleotide abnormalities. Gynaecological cancers may occur as sentinel events for Lynch syndrome in women. Hence, there is an opportunity to screen for and diagnose LS in women before other cancers occur, permitting cancer surveillance programmes, preventative measures, cascade testing of at-risk relatives and influencing treatment options, such as immune checkpoint blockade. Screening of cancer samples for LS usually involves MMR IHC and/or MSI testing, with MLH1 promoter methylation testing to distinguish sporadic MLH1-negative cancers, with subsequent germline sequencing. MMR IHC analysis can identify the specific protein that is lost or abnormal, indicating the likely mutated gene. MMR proteins form heterodimers, MLH1 – PMS2 and MSH2 – MSH6, that are stable. However, MMR proteins are unstable in the unpaired state. Although MLH1 and MSH2 can form stable heterodimers with other proteins, PMS2 and MSH6 only dimerise with MLH1 and MSH2 respectively, affecting interpretation of the patterns of loss or abnormality, with this interpretation best performed by an experienced pathologist and with high quality MMR IHC staining requiring participation in a NEQAS scheme.

◆ Case Presentation: Spectrum of MMR Deficient Cases in Routine Practice

Ⓟ AD Attygalle

Royal Marsden Hospital, London, UK

Immunohistochemical expression of mismatch repair proteins is a reliable screening test for Lynch Syndrome and also in the detection of sporadic cases of mismatch repair deficiency and is therefore used routinely as part of the histological work-up of all cases of endometrial carcinomas and endometriosis associated ovarian carcinomas. It is also pivotal in the algorithmic approach used in the clinical application of the TCGA integrated genomic classification of endometrial carcinomas, identifying the hypermutated MSI high subgroup. Accurate interpretation of immunohistochemistry is crucial and is dependent not only on good technical optimisation but also the awareness of pitfalls related to fixation and background/non-specific staining. The cases presented will illustrate the spectrum of mismatch repair deficiency in practice and also highlight difficulties associated with interpretation.

◆ A Day in the Life of a Histopathologist

Ⓟ SC Lishman

North West Anglia NHS Foundation Trust, Peterborough, UK

Pathology is made up of numerous different specialties, the largest of which is histopathology. As a histopathologist, no two days are ever the same. My day might include performing a post mortem examination for the Coroner to find out why someone has died, giving evidence at an inquest, dissecting entire organs that have been removed in the operating theatre, presenting cancer cases at a multi-disciplinary team meeting or, the bulk of my work, examining slides under the microscope to make a diagnosis. I request and interpret immunohistochemical and molecular tests, teach biomedical and medical students and doctors in training, and contribute to research and quality improvement processes. I also give talks to schools and the public about pathology, represent the specialty on national committees and attempt to influence policy makers to ensure that there is appropriate investment in pathology services. My talk will concentrate on the diagnostic work of a histopathologist but you will get a glimpse of the other fascinating roles that exist in the specialty and beyond.

◆ Stromal Tumours of the Bladder

Ⓟ JH Shanks

The Christie NHS Foundation Trust, Manchester, UK

A large variety of mesenchymal tumours occur in the bladder. An area of particular diagnostic difficulty is interpretation of spindle cell lesions and distinction of sarcomatoid carcinoma and sarcoma from the group of lesions referred to as pseudosarcomatous myofibroblastic proliferation (PMP). The presentation will focus on distinction of PMP/IMT from sarcomatoid carcinoma and spindle cell sarcomas. Look for areas of nuclear hyperchromasia with pleomorphism, often with high cellularity. If possible, use wide sampling to seek any typical carcinoma merging with the lesion or carcinoma in situ. Mitotic figures may be present in bladder IMT/PMP, but not atypical mitoses. Necrosis is common on the ulcerated surface but is not specific.Deep necrosis away from the surface is rare in bladder IMT/PMP but if present in isolation, with otherwise typical features, is insufficient for malignancy. Sarcomatoid carcinomas of bladder frequently show minimal/focal cytokeratin positivity. Blocks with the most 'epithelioid' areas should be selected for work-up. Broad spectrum cytokeratins such as MNF116 and AE1/3 are not specific, and paradoxically are more likely to be extensively positive in IMT/PMP of bladder, in contrast to often very limited staining in sarcomatoid carcinoma. p40 and high molecular weight cytokeratins (CK5/6 and/or 34betaE12) are more specific for sarcomatoid carcinoma, if positive. Positive ALK-1 immunohistochemistry in the context of a bladder spindle cell lesion is strong supportive evidence of IMT/PMP, providing rhabdomyosarcoma is excluded by an appropriate panel. Approximately 50% of bladder IMT/PMPs are ALK-1 positive by immunohistochemistry, so a negative does not exclude IMT/PMP. FISH with breakapart probes demonstrates a signal pattern consistent with ALK-1 gene rearrangement (at 2p23) in a similar proportion. Rhabdomyosarcoma should be considered for a mesenchymal bladder lesion in children; myogenin and MyoD1 should be included in in the panel.

◆ Colorectal Cancer: Phenotype vs. Genotype

Ⓟ NP West

University of Leeds, Leeds, UK

Colorectal cancer is the fourth most common cancer in the UK and the second most common cause of cancer-related mortality. Patient outcomes have significantly improved year on year due to screening and advances in treatment to include better imaging, surgery and oncology. Pathologists have played a major role in improving outcomes over many years through describing the tumour phenotype from macroscopic and microscopic interpretation of the resection specimen. In recent years, advances in molecular pathology have led to additional tests including genotyping being introduced into routine clinical practice to facilitate stratified medicine. Many molecular markers proposed in the literature never make it into routine practice due to a failure to validate in larger prospective studies or providing only limited additional clinical value. It is critical when evaluating any new potential biomarker that it gives significant added value to the current gold standard and is economically viable. This talk will summarise the important phenotypic and genotypic markers available to pathologists reporting colorectal cancer specimens and suggest which ones are the most valuable.

◆ Tools You Need to Analyse Genomics

Ⓟ HM Wood

University of Leeds, Leeds, UK

Background: Genomic data, and similar high throughput information, are increasingly being used in a pathological setting, both for research and as a diagnostic tool. The volumes of data produced makes analysis using standard office software impossible. Whilst there is a plethora of specialist tools available, it can be difficult to decide which is the best tool for each step, and daunting to learn some of the steps for people with little or no coding or command line experience.
Rationale behind pipelines: All sequence data goes through a number of similar steps, such as adapter trimming and alignment before more specialised steps such as variant calling or read counting. Similarly, array-based methods have some steps in common with all tools, and some which are specific. It is important to understand the rationale behind these steps individually so that an informed decision can be made as to how to construct an analysis pipeline.
Available resources: A number of bioinformatic solutions are available, which are designed for a variety of methodologies and require different levels of informatic experience. Some, such as the Agilent Surecall, are designed around one or a few specific assays. Others, such as the proprietary Illumina Basespace, or the open source Galaxy platform are more modular in nature and allow users to build pipelines in a familiar mouse-based computing environment. Other pipelines can be built from individual packages assembled in a command line environment, which are harder to learn, but allow for greater flexibility and automation. Most pipelines can be run on local machines or in a cloud computing infrastructure. Once data has been processed, high level analysis can be carried out using standard software such as excel or SPSS, or more specialised tools such as R or MATLAB. Through understanding the processes needed to analyse their data and the available tools, users can build analysis pipelines which best suit their particular situation.

◆ TILs: The Next Morphological Biomarker in Breast Cancer for Daily Practice Use?

Ⓟ R Salgado

Peter MacCallum Cancer Centre, Mebourne, Australia

The assessment of Tumour Infiltrating Lymphocytes (TILs) is gaining importance as a prognostic marker in breast cancer. Recently, the 2019 St Gallen Breast Cancer Conference as concluded that TILs should be routinely reported in TNBC and TILs will be included also in the 2019 WHO/IARC Blue Book edition on Breast Tumour classification. High TILs are associated with a better outcome and a better response to neoadjuvant therapy in Triple-negative and HER2 positive breast carcinomas, as well as having strong prognostic value in improving estimates of distant recurrence-free survival , disease-free and overall survival in early-stage TNBC treated with standard adjuvant/neoadjuvant chemotherapy (Level 1B evidence). This is based on an evaluation by pathologists using H&E stained glass slides at time of diagnosis (pre-treatment and in the residual disease post neoadjuvant chemotherapy). Their quantification is done on H&E tissue sections during diagnosis procedure and follows international recommendations (www.tilsinbreastcancer.org). Development of computational pathology and machine learning methods in this area is very promising. Clinical utility using TILs as a biomarker for selection of patients for treatment with immune-checkpoint-inhibition is ongoing. TILs evaluated on an HE as an alternative to classical biomarkers such as PDL1 is promising based on phase I and II trial data. TILs are used as a stratification factor in clinical trials and should be included in all studies involving or evaluating prognosis. At the current lecture the international consensus scoring recommendations, pitfalls on TIL-assessment and how to mitigate these pitfalls, will be elaborated on (see www.tilsinbreastcancer.org). Evidence will be presented that the current recommendations can be extrapolated to other tumour settings, such as lung cancer.

◆ Breast Digital Pathology in Day-to-Day Practice

Ⓟ RA Millican-Slater

Leeds Teaching Hospitals Trust, Leeds, UK

As described by the Royal College of Pathologists, "digital pathology has the potential to improve patient care and support the pathology workforce by making the diagnosis and monitoring of disease much more efficient." At Leeds Teaching Hospitals NHS Trust we have been scanning almost all the glass slides for breast specimens since January 2017, and since May 2017, I have been reporting the vast majority of my workload using scanned images and using a digital microscope rather than glass slides and the traditional light microscope. This shift in practice brings huge benefits and opens up the potential for exciting developments, though is not without its challenges. I will describe my personal experience in moving to using digital pathology for day-to-day diagnostic practice, including the validation process, the advantages of using a digital system, the difficulties in adopting a digital workflow, key lessons learned and future opportunities.

◆ Combining Clinical and Academic Training in Pathology

Ⓟ JL Griffin

Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK

Histopathology training is an ideal environment in which to pursue a combined clinical academic career: A focus on applied science, daily use of techniques used in research and civilised working hours all contribute to histopathologists being well placed to work in basic, translational and clinical research. As personalised medicine becomes an established part of clinical care, the need for research-active histopathologists is greater than ever, as are the opportunities to get involved in this interesting and demanding career. In this talk I will discuss the routes into academic pathology, the broad range of opportunities that are available and how to balance research with clinical training.

◆ The Role of Molecular Pathology in Cancer Care

Ⓟ RJ Byers

University of Manchester, Manchester, UK

Pathology is a constantly evolving field and clinical discipline due to advances in understanding of disease, technology platforms and requirements for improved patient care. This is well illustrated by the increasing role of molecular pathology in cancer care. Haematopathology, which deals with diagnosis of lymphoma and leukaemia has led the field in application of molecular pathology, resulting in significant advances in our understanding of the disease processes underlying these cancers, the development of targeted treatment and improved patient outcomes. More recently similar advances have been made in lung, breast and colorectal caner. I will give an overview of the above themes in the clinical role of molecular pathology in patient care.

◆ IgG4-Related Disease in the Pancreatobiliary System: How Good is Histopathology as the Gold Standard of Diagnosis?

Ⓟ BH Haugk

Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK

IgG4-related disease (IgG4-RD) is a rare, multiorgan, fibroinflammatory condition that most commonly affects the pancreatobiliary system and responds well to corticosteroid treatment. It clinically presents with tumefactive lesions that can mimic neoplasms on imaging and therefore its correct diagnosis is vital in preventing unnecessary, potentially life changing surgery. Type 1 autoimmune pancreatitis is the commonest presentation of IgG4-RD affecting approximately 60% of patients. It can be associated with IgG4-related sclerosing cholangitis, IgG4-related hepatopathy and/or IgG4-related cholecystitis but isolated hepatobiliary presentations are rarely seen. IgG4-RD is an organ-destructive disease with characteristic histopathological features: 1. Dense lymphoplasmacytic inflammatory infiltrate, often with eosinophils, 2. Fibrosis, at least focally with a storiform pattern, 3. Obliterative phlebitis, as well as immunohistochemical evidence of tissue specific levels of increased IgG4 positive plasma cells with an IgG4+/ IgG+ plasma cell ratio >40%. Confident pathological diagnosis may be possible on resection specimens but confirming IgG4-RD in small biopsies poses a great challenge and mere IgG4 staining is neither diagnostic nor predictive of IgG4-RD. Histopathology remains nevertheless an important component in the diagnosis of IgG4-RD in this setting. Reaching a diagnosis requires striking a balance between strict application of diagnostic histological criteria of IgG4-RD in biopsies and close correlation with pre-treatment clinical features including specific imaging findings, multiple organ involvement and elevated serum IgG4. The crucial role of the histopathologist will often lie in the thorough exclusion of malignant neoplasms in biopsy material but also reviewing potential previous archival histopathological specimens for presence of IgG4-RD. A multidisciplinary team approach is critical in facilitating best patient care.

◆ Recent Advances in Hepatocellular Carcinoma

Ⓟ DG Tiniakos

Institute of Cellular Medicine, Newcastle upon Tyne, UK

Recent classification of hepatocellular carcinoma (HCC) is based on morphology and underlying molecular alterations recognising two main HCC subgroups, the proliferative with worse prognosis (expression of stem cell markers, *TP53* mutations, *FGF19* amplification) and the non-proliferative (activation of *JAK/STAT* pathway, *CTNNB1* activating mutations). New subtypes of HCC have been introduced: massive macrotrabecular HCC (5-10% of HCC, high serum AFP, large size, trabeculae >6-cell thick in >50% of the tumour, predictor of HCC recurrence, *TP53* mutations and *FGF19* amplification); steatohepatitic (steatosis, ballooning or Mallory–Denk bodies, fibrosis and inflammatory foci, JAK/STAT pathway activation, more common in patients with the metabolic syndrome and steatohepatitis in the background liver); chromophobe HCC (5% of HCC, related to HBV infection, alternative lengthening of telomere -ALT phenotype by telomere flurorescent *in situ* hybridsation (FISH); other less common entities. A specific fusion transcript, DNAJB1-PRKACA, has been identified in fibrolamellar carcinoma (FLC). The resulting chimeric kinase functions as a driver of carcinogenesis in FLC and FISH for the PRKACA rearrangement is useful for confirming diagnosis. An international multidisciplinary group has proposed a consensus terminology for primary liver carcinomas with both hepatocytic and cholangiocytic differentiation. The diagnosis of combined hepatocellular carcinoma-cholangiocarcinoma (cHCC-CCA) should be based on morphology using routine haematoxylin and eosin and not on immunophenotype. The morphology of intermediate cell carcinoma is distinct and immunohistochemistry for hepatocytic (i.e. HepPar1) and cholangiocytic markers (i.e. keratin 19) is required to highlight its mixed HCC-CCA differentiation. Cholangiolocellular carcinoma is a distinct molecular entity that is now classified within cholangiocarcinoma.

◆ The Role of Liver Biopsy in an Era of Expanding Non-Invasive Assessment

Ⓟ SE Davies

Cambridge University Hospitals NHS FT, Cambridge, UK

The role of liver biopsy has undergone a major shift from the original indications some 50 years ago, when it would have been primarily used for diagnosis. It is now infrequent that clinicians don't have a good idea of the diagnosis based on serology of autoantibodies, including the expanding catalogue of 2nd order antibodies in autoimmune processes with atypical presentations, of viral antibodies (hepatitis A to E being routinely available) and genetic studies. The commonest risk factors are environmental, Fatty Liver Disease either related to alcohol or otherwise, primarily Insulin Resistance and may be relatively easily ascertained. And so the role of biopsy has evolved to one of attempting to Grade the severity of the process and Stage the extent, usually by the amount of fibrosis, with a view to prognosis and patient management. We are also in an era where non-invasive methods of assessing the stage of disease are becoming readily available and reliable, some based on blood tests and complex algorithms, others with assessment of liver stiffness as an indication of fibrosis. In reality, there is often MORE than 1 aetiology possible and the role of biopsy is to establish which may be the dominant factor. Partly this reflects the number of people in society at risk of Fatty Liver Disease increasingly being picked up with abnormal liver biochemistry or who have this as a second pathology within the biopsy. There are often multiple possible causal agents for acute derangement of liver function, particularly with the use of drugs in oncology patients or on immunosuppression. The overall incidence of dual pathology present in biopsies has increased, making the assessment more complex and challenging to the pathologist. Awareness of the clinical scenario and question being asked is essential to interpretation of a liver biopsy.

◆ Autoimmune Hepatitis: The Role of Histopathology in Clinical Diagnosis and Management

Ⓟ SG Hubscher

University of Birmingham, Birmingham, UK

Liver biopsy continues to play an important role in the diagnosis and management of patients with autoimmune hepatitis (AIH). The histological assessment of liver biopsies from patients known or suspected to have AIH involves two main components:
1: Establishing a diagnosis of AIH. In the absence of a specific diagnostic test for AIH, liver biopsies are frequently obtained at the time of first presentation. Histological assessments include identifying the presence of typical or compatible features that would support a diagnosis of AIH and the absence of features that might suggest an additional or alternative diagnosis (e.g. biliary disease or fatty liver disease). Typical features include lymphoplasmacytic inflammation of portal tracts, interface hepatitis, hepatocyte rosettes and emperipolesis. These features may be helpful in distinguishing AIH from other diseases associated with portal/periportal hepatitis. Cases with an acute presentation typically have prominent lobular inflammation and may be difficult to distinguish from other causes of acute lobular hepatitis (e.g. viral agents and drugs).
2: Assessment of disease severity. This includes the assessment of inflammatory activity and fibrosis, both of which have implications for treatment and prognosis. The severity of interface hepatitis in baseline biopsies predicts the subsequent development of fibrosis. In patients who have achieved biochemical remission following treatment with immunosuppressive agents, the presence of persistent interface hepatitis is associated with an increased risk of relapse if immunosuppression is withdrawn. In patients with an acute presentation, the presence of extensive bridging or panacinar necrosis is associated with an increased risk of acute liver failure. Between 25% and 33% of patients have cirrhosis at presentation, including some patients with an acute presentation. Such cases may be less responsive to immunosuppression and are at risk of developing HCC.

◆ Hypoxia

Ⓟ CW Pugh

University of Oxford, Oxford, UK

The variable levels of oxygen encountered in different tissues in health and disease will be described, including a discussion of the association between tumour hypoxia and outcomes. The hypoxia-inducible factor signalling pathway will be reviewed, including a brief discussion of the roles of different components in physiological and pathological responses. Effects of hypoxia and hypoxia-signalling on the immune system in both human tumours and model systems will be outlined. Some suggestions will be made on how this information might be used both in the classification and therapy of diseases.

◆ The Interplay Between Cell Stress, Cell Death and Inflammation

Ⓟ SJ Martin

Trinity College Dublin, Dublin, Ireland

Inflammation is initiated in response to Infection, Injury or Cell Stress, that all share the ability to elicit the production of an array of cytokines, chemokines and other factors that recruit cells of the innate immune system and also initiate wound repair. While it is very well established that conserved components of infectious agents, called PAMPs (pathogen-associated molecular patterns), and molecules released by necrotic cells, called DAMPs (damage-associated molecular patterns), promote inflammatory responses, it is less well appreciated that conditions which provoke Cell Stress (such as misfolded protein-induced ER stress, mitochondrial depolarization, heat shock, DNA damage) can also instigate inflammatory cytokine production in a manner that is poorly understood at present. Receptors for TRAIL and FasL have been dubbed 'death receptors' as these receptors can act as potent initiators of apoptosis in many cell types. As a consequence, 'death receptor' signaling has been studied almost exclusively in the context of cell death outcomes. However, TRAIL and FasL can also induce NFκB-dependent expression of pro-inflammatory cytokines, which can engage multiple facets of the immune system (Cullen et al., 2013; Cullen and Martin, 2015). We have recently shown that TRAIL and Fas receptor engagement leads to the assembly of an NFκB-activating 'FADDosome' complex where caspase-8 unexpectedly plays a critical scaffold role in the process leading to inflammatory cytokine production (Henry and Martin, 2017). Thus, in addition to their well-known roles as a 'death ligands', TRAIL and FasL can promote inflammation in certain settings. Here, I will discuss recent data from our laboratory that suggest that cell stress leads to inflammation via death receptor upregulation and activation.

◆ Robotic Surgery in Head and Neck Cancer: Implication for Pathologists

Ⓟ M Robinson

Newcastle University, Newcastle upon Tyne, UK

Trans-Oral Robotic Surgery (TORS) was approved by the FDA in 2009. Over the past decade surgical robotic systems have been acquired by the NHS, mainly for urological surgery, however, UK head and neck surgeons have taken the opportunity to utilize the technology for the benefit of their patients. There are currently around 15 units providing a TORS service in England. Whilst the surgical methods have evolved, there has been little consideration about the pathological assessment of the surgical specimens and a lack of understanding around the oncological principles that underpin minimally invasive surgical techniques in the head and neck region. This presentation will address the pathological issues encountered during the handling of TORS specimens. The challenges include identifying anatomical boundaries, appropriate block selection, assessment and interpretation of resection margins, the use of intra-operative frozen sections, the assessment of the tongue base mucosectomy for patients with head and neck cancer of unknown primary, and TORS for recurrent disease. The presentation will conclude with emerging innovations, such as the intelligent-Knife (i-Knife) to guide surgical excision and the potential of step serial sections to identify sub-clinical HPV-related oro-pharyngeal squamous cell carcinomas.

◆ Update in Salivary Gland Pathology: New Terminology and Molecular Diagnostics

Ⓟ RHW Simpson

University of Calgary, Calgary, Canada

Several primary salivary neoplasms have been shown to have characteristic molecular genetic abnormalities. These are increasingly important in diagnosis and probably soon as the basis for specific therapy. There is also a strictly limited role as prognostic markers in some cases. The best established neoplasms with specific molecular signatures are mucoepidermoid, adenoid cystic, (mammary analogue) secretory and hyalinising clear cell carcinomas, with rearrangements of MAML2, MYB, ETV6 and EWSR1 respectively. There are usual gene partners and for example, in mucoepidermoid, most show a CRTC1-MAML2 fusion, but in a minority, this is CRTC3-MAML2. Analogous genetic variants have been described in the other carcinomas. In addition to the malignancies, about 70% of pleomorphic adenomas show abnormalities of PLAG1 or HMGA2 genes, although this is of limited value in everyday practice. A second group of other carcinomas also have genetic abnormalities, although the value of these findings is yet to be established; in polymorphous (low grade) adenocarcinoma, most display a recurrent E710D hotspot somatic mutation within the catalytic loop of the kinase domain of PRKD1, and in cribriform adenocarcinoma (CATS or CASG) 80% of cases were found to have rearrangements of the PRKD1-3 genes. Recently, a HTN3-MSANTD3 fusion was demonstrated in a subset of acinic cell carcinomas. A third group is that of neoplasms that have well established molecular signatures, and arise in the salivary glands only exceptionally rarely – these include NUT carcinoma, desmoplastic small round cell tumour and adamantinoma-like Ewing's sarcoma. All these genetic rearrangements have allowed us better to define various entities morphologically and for example, hyalinising clear cell carcinoma is no longer a diagnosis of exclusion. The 2017 WHO Classification recognises some of these developments, but this is a fast developing field and the classification will require updating again fairly soon.

◆ CTCF Maintains Regulatory Homeostasis of Cancer Pathways

Ⓟ SJ Aitken[1]; X Ibarra-Soria[1]; E Kentepozidou[2]; P Flicek[2]; C Feig[1]; JC Marioni[2]; DT Odom[1]

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CTCF binding to DNA helps partition the mammalian genome into discrete structural and regulatory domains. Complete removal of CTCF from mammalian cells causes catastrophic genome dysregulation, likely due to widespread collapse of 3D chromatin looping and alterations to inter- and intra-TAD interactions within the nucleus. In contrast, *Ctcf* hemizygous mice with lifelong reduction of CTCF expression are viable, albeit with increased cancer incidence. Here, we exploit chronic *Ctcf* hemizygosity to reveal its homeostatic roles in maintaining genome function and integrity. We find that *Ctcf* hemizygous cells show modest but robust changes in almost a thousand sites of genomic CTCF occupancy; these are enriched for lower affinity binding events with weaker evolutionary conservation across the mouse lineage. Furthermore, we observe dysregulation of the expression of several hundred genes, which are concentrated in cancer-related pathways, and are caused by changes in transcriptional regulation. Chromatin structure is preserved but some loop interactions are destabilised; these are often found around differentially expressed genes and their enhancers. Importantly, the transcriptional alterations identified *in vitro* are recapitulated in mouse tumours and also in human cancers. This multi-dimensional genomic and epigenomic profiling of a *Ctcf* hemizygous mouse model system shows that chronic depletion of CTCF dysregulates steady-state gene expression by subtly altering transcriptional regulation, changes which can also be observed in primary tumours.

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This abstract has been published previously: "Sarah J. Aitken, Ximena Ibarra-Soria, Elissavet Kentepozidou, Paul Flicek, Christine Feig, John C. Marioni & Duncan T. Odom. CTCF maintains regulatory homeostasis of cancer pathways. *Genome Biol.* 2018 Aug 7;19(1):106. doi: 10.1186/s13059-018-1484-3."

◆ Colorectal Cancer Biomarkers: From Molecular Modelling to Immunohistochemistry

Ⓟ GI Murray

University of Aberdeen, Aberdeen, UK

Colorectal cancer is one of the commonest types of cancer and it has been recognised for many years that there is a requirement to identify biomarkers of this type of tumour which can provide prognostic information additional that obtained by careful histopathologic assessment. This lecture will describe the discovery and validation of colorectal cancer biomarkers based on the development of monoclonal antibodies to tumour associated proteins. Initial studies used small patient cohorts now much larger patient cohorts are available. The sophistication of the molecular approach has evolved embracing a range of molecular tools and technologies for studying proteins. Early studies developed monoclonal antibodies to invasion associated proteins in particular matrix metalloproteinases. Amino acid sequence alignment combined with low resolution 3D protein molecular modelling was used to identify likely antigenic regions that could be targeted for the development of sequence specific monoclonal antibodies. Through this approach matrix metalloproteinase-1 was as identified as a marker of poor prognosis in colorectal cancer. Current studies now utilise a range of sophisticated molecular tools to identify potential protein targets. Amino acid sequence homology, antigenic prediction, protein modelling have all contributed to developing a robust approach to identify tumour associated proteins for which monoclonal antibodies can be developed. These studies have identified the brown fat associated proteins as a novel pathway in colorectal cancer and uncoupling protein 1 as a prognostic biomarker in both discovery and validation cohorts of colorectal cancer.

◆ Update on Lung Cancer Staging

Ⓟ AG Nicholson

Royal Brompton Hospital, London, UK

There have been several changes in the eighth TNM that impact on the pathological staging of lung cancer.
1: In relation to the T category, from 1 to 5 cm, each centimetre increase in cancer diameter is associated with worsening survival (T1a-c, 2a,b). Cancers greater than 5 cm but less than or equal to 7 cm are now staged as T3, and those greater than 7 cm as T4. T2 classification is also used for tumors, breaching the visceral pleura, involving the main bronchus and tumors associated with atelectasis or obstructive pneumonitis that extends to the hilar region, either involving either part of the lung or the whole lung. Involvement of the diaphragm has a T4 prognosis. Invasion of the mediastinal pleura was seldom used and is has been discontinued.
2: For sub-solid nodules, 2011, new entities of adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), and lepidic predominant adenocarcinoma were defined. leading to recognition of introduction of a Tis(AIS) classification for adenocarcinoma in situ (Tis(AIS)) and T1mi for minimally invasive adenocarcinoma. For tumours with lepidic growth, only the invasive size of the tumour should be used for the T size. As the amount of lepidic tumour may potentially be underestimated grossly, evaluation of cancer size may require re-examination of the specimen and careful correlation with microscopic and radiographic findings.
3: For multiple tumours, staging overall has not changed, but there is additional notation that recognizes (a) separate primary lung cancers presenting as predominantly ground-glass opacities on imaging with typically non-mucinous adenocarcinoma showing lepidic predominant morphology, and (b) pneumonic presentation on imaging that typically correlates with invasive mucinous adenocarcinoma.
4: Other areas (R category (e.g. R1(cy+) , subdivision of N) also have proposed changes for data collection in relation to the 9th TNM, and the impact of these on pathological staging will be discussed

◆ Molecular Biology of Mesothelioma-Implications for Diagnosis and Management

Ⓟ F Galateau Salle

Cancer Center Leon Berard, Lyon, France

Malignant mesothelioma is a rare cancer less than 0,3% of all cancers, highly lethal, histologically extremely heterogeneous cancer, resistant to the majority of conventional chemotherapies. This disease is largely linked to asbestos exposure in more than 80% of the cases in men, occurring with a long delay of latency (30-40 years after initial exposure). The major direct damage of asbestos fibers on mesothelial cells and the chronic inflammatory response (macrophages and other immune cells) with release of oxygen species may be an important factor in the carcinogenesis of malignant mesothelioma. More recently, mesothelioma was reported to be associated with inherited genetic mutations in less than 5% and can occurs in family being part of the BAP1 cancer associated syndrome. The last WHO classification in 2015 of malignant diffuse or localized mesothelioma reported that the most prevalent type was subdivided in epithelioid (80%) sarcomatoid (up to 10%) and biphasic (in 10-15%) composed of epithelioid and biphasic morphologies with at least 10% of each component suggesting a role for epithelial to mesenchymal transition at the molecular level. All these characteristics make this tumour extremely original molecularly compared to other cancers. However, since recently there was a very limited use of molecular testing in malignant mesothelioma compared to lung cancer explaining the weaknesses of knowledge on molecular biology for diagnosis and treatment of this dramatic disease. Then, analysis based on NGS or Sanger sequencing were reported by Bueno et al, identifying recurrent somatic alterations in BAP1, Nf2 and CDKN2A(p16), CUL1, TP53, SETDB1 with additionnal findings reported by Hmeljak et al from TCGA analysis showing a strong expression of the immune checkpoint gene VISTA with implications for immune response to immunotherapy. Alcala et al, found that the molecular profile of MPM was better explained by a continuous spectrum based on an immune an vascular pathway.

◆ Diagnosing Interstitial Lung Disease Is No Longer "Business as Usual": A Contemporary Diagnostic Approach to Fibrosing Interstitial Pneumonias

Ⓟ BT Larsen

Mayo Clinic Arizona, Scottsdale, USA

Purpose: Idiopathic pulmonary fibrosis (IPF) is a syndrome characterized by progressive lung scarring, manifest as the usual interstitial pneumonia (UIP) pattern. Recent therapeutic advances have made distinction of IPF from all other fibrosing interstitial pneumonias essential. Per consensus criteria, diagnosis of IPF no longer requires a biopsy in an appropriate context if a UIP pattern is seen radiologically. In current practice, lung biopsies are typically reserved for patients having indeterminate clinical or imaging findings. Consequently, the frequency of encountering biopsies with other fibrotic patterns may have increased in recent years.
Methods: To update pathologists on fibrotic interstitial pneumonias, IPF and its common mimics will be reviewed, including connective tissue disease-associated interstitial lung disease, chronic hypersensitivity pneumonitis, and other less common causes of diffuse lung fibrosis.
Results: It is incumbent on pathologists to be aware of the shifting landscape in pulmonary pathology and the clinical implications of histological diagnoses in the current era. In addition, pathologists must recognize the critical role of multidisciplinary discussion in the diagnostic process. For IPF and each of its mimics, histological characteristics will be reviewed and clinical and radiological findings will be discussed, emphasizing findings that can be used to increase diagnostic certainty and enable distinction of IPF from other entities. Throughout this review, the clinical implications of histological diagnoses will be emphasized, and current treatment approaches will be reviewed.
Conclusion: Often, clues in the lung biopsy may offer the first suggestion of a non-IPF diagnosis when lung fibrosis is encountered, but clinical and radiological information must also be incorporated to arrive at the best diagnosis. Accurate classification of fibrosing interstitial pneumonias is essential for proper treatment selection.

◆ The Human Protein Atlas: Implications for Human Biology and Precision Medicine

Ⓟ C Lindskog

Uppsala University, Uppsala, Sweden

In the evolving era of "big data", integration of datasets from different omics technologies, such as genomics, transcriptomics and proteomics have received increased attention. The Human Protein Atlas database (www.proteinatlas.org) with >200.000 visits per month, constitutes a comprehensive open-access knowledge resource for spatial localisation of proteins in organs, tissues, cells and organelles. The mapping is based on genome-wide mRNA expression data, which is summarised on the interactive webpage and used for categorisation of all human genes based on expression level and tissue distribution. The analysis is combined with tissue microarray-based immunohistochemistry covering all major normal human organs and cancer types, and a large effort is put into enhanced antibody validation strategies. In 2017, a new Pathology Atlas was released, based on genome-wide expression data from the Cancer Genome Atlas. RNA-Seq data and clinical metadata from 8,000 individual patients corresponding to 17 major cancer types was used for determining the correlation between RNA expression levels and overall survival time for each gene in each cancer type. The new data, together with antibody-based protein expression data from the corresponding cancer types, opens up for pursuing better diagnostic schemes and designing personalised cancer treatment regimes.

◆ Next Generation Immunohistochemistry: Considerations for the Practising Pathologist

Ⓟ JM Ziai

Genentech, Inc., South San Francisco, CA, USA

Highly multiplexed methods to profile tissues' protein and RNA content are becoming increasingly common. While not currently part of routine practice, the "next generation" of immunohistochemical (IHC) methods will increasingly impact basic and translational research as well as diagnostic practice in the coming years. The surgical pathologist – whether a diagnostician, research collaborator or primary investigator – must therefore develop a sophisticated understanding of the chemistry, function, and composition of various multiplexing methods as well as their respective strengths and limitations to continue to serve as an effective consultant to both researchers and clinicians. Multiple methods that enable both "low" (<6) and "high" (>6) level multiplexed IHC are currently available for use. However, use is often limited to technology access programs and can be cost-prohibitive even for small studies. The initiation, design, sample selection, and data interpretation of a multiplexed IHC study must therefore be carefully considered. This lecture will provide an overview of multiplexed IHC methods that are available today with focus on both "low" level methods (e.g. tyramide signal amplification) as well as "high" level methods, particularly mass spectrometry-based methods (imaging mass cytometry (IMC), multiplex ion beam imaging (MIBI)) and NanoString Digital Spatial Profiling (DSP). Learnings from pilot studies with both DSP and MIBI/IMC on such issues as method chemistry, sensitivity, and reproducibility will be shared and recommendations for application, experimental design and interpretation of highly multiplexed IHC from these platforms will be summarized.

◆ Receptor Tyrosine Kinase Signalling in the Absence of Kinase Activity and Cancer of Non-Genetic Origin

Ⓟ JE Ladbury

University of Leeds, Leeds, UK

FGFR2-expressing cancer cells with low concentrations of the adaptor protein Grb2 show high prevalence for metastatic outcome. In non-stimulated cells the SH3 domain (and not the SH2 domain(s)) of Plcγ1 directly competes for a proline-rich binding site at the very C-terminus of FGFR2 with the C-terminal SH3 domain of Grb2. Reduction of Grb2 concentration permits access of Plcγ1 to the receptor. Recruitment of Plcγ1 in this way is sufficient to up-regulate phospholipase activity. This results in increased cell motility and promotion of cell invasive behaviour in the absence of extracellular receptor stimulation. Therefore metastatic outcome can be dictated by the constitutive competition between Grb2 and Plcγ1 for the phosphorylation-independent binding site on FGFR2. Since the majority of receptor tyrosine kinases have proline-rich sequences in their C-termini, the possibility of a second tier of signal transduction in the absence of growth factor stimulation, or kinase-activating mutations emerges – leading to cancer of non-genetic origin.

◆ The Fourth Edition of the WHO Classification of Skin Tumours: Classification of Melanoma

Ⓟ DM Massi

University of Florence, Florence, Italy

The recent WHO Classification of Skin Tumors published in 2018 (1) has introduced a novel multidimensional approach to the classification of melanoma defining nine biologically distinct pathways to melanoma development. These pathways take origin from the integrated taxonomy of melanocytic neoplasms which encompasses the cell of origin (epithelium and non-epithelium associated melanocytes), the pathogenetic role of ultraviolet radiation and skin pigmentation, and the complex genomic and molecular alterations whose knowledge and mechanisms have been deeply and remarkably enriched over the past years. On a molecular basis, melanoma can be considered as a group of biologically distinct lesions that develop through different mutational pathways. Considering the integrated taxonomy further developed for the WHO Classification of Skin Tumors, fourth edition, published in 2018 (1), the following pathways have been defined:
Pathway I: Low-Cumulative Solar Damage (CSD) Melanoma (including Superficial Spreading Melanoma and low-CSD Nodular Melanoma)
Pathway II: High-Cumulative Solar Damage (CSD) Melanoma (including Lentigo Maligna Melanoma and high-CSD Nodular Melanoma)
Pathway III: Desmoplastic Melanoma
Pathway IV: Malignant Spitz Tumor
Pathway V: Acral Melanoma
Pathway VI: Mucosal Melanoma
Pathway VII: Melanoma arising in Congenital Nevus
Pathway VIII: Melanoma arising in Blue Nevus
Pathway IX: Uveal Melanoma
Each pathway is characterized by specific epidemiology, clinical and histopathological features, pattern of metastasis, etiopathogenetic role of UV radiation, predisposing germ-line alterations, somatic mutations, and mutator mechanisms. The main molecular and histopathological features of the different pathways will be critically discussed.
References: 1. Elder DE, Massi D, Scolyer RA, Willemze R, Editors. WHO Classification of Skin Tumours. Fourth Edition, Lyon: IARC; 2018.

◆ Determining Risk Status from Pathology, Genetics and Outcome Data in Cutaneous Squamous Cell Carcinoma: A National Project

Ⓟ PJ Craig

Gloucestershire Hospitals NHSFT, Cheltenham, UK

Primary cutaneous squamous cell carcinoma (cSCC) has been studied little compared to other cancers perhaps due to a lack of good epidemiological and outcome data, and a lack of effective treatment options for those with advanced disease. Recently, in collaboration with Public Health England (PHE), incidence data for primary cutaneous SCC per year incorporating data from histopathology reports has shown an incidence of over 25500 per annum (Venables ZC et al. Nationwide Incidence of Metastatic Cutaneous Squamous Cell Carcinoma in England. JAMA Dermatol. 2018 Nov 28). Furthermore they were able to assess the incidence of metastatic cSCC by matching primary and secondary cancers in the same patient. Metastatic disease occurred in 2.4% of cSCC in males and 1.1% of females with an increased risk on the lip, ear, age >90 and immunosuppression, and 70% occurred within the first 2 years of diagnosis of the primary cSCC. A further study from the same group has shown an increase in incidence of 5% per annum for both cSCC and basal cell carcinoma. Indeed, some have predicted a doubling in incidence for cSCC in the next 25 years, due to an ageing population and increased UV exposure. Given this disease and management burden, cSCC has been identified as a research priority by the British Association of Dermatologists (BAD). Using this data in conjunction with PHE, a research group (UK Kertatinocyte Collaborative) has been set up including histopathologists to create a virtual tissue bank of these cases to produce an extensive, valuable research resource so the research community including pathologists can apply to use this material. The project will aim to use pathology, genetics and outcome data to determine risk status and guide management and new therapies.This will require collaboration from pathologists in confirming the diagnosis and sourcing the paraffin blocks. This lecture will discuss the project and epidemiology, pathology, genetics and current therapies for cSCC.

◆ Newer and Unusual Forms of Oesophagitis and Gastritis

Ⓟ K Sheahan

St Vincent's University Hospital, Dublin, Ireland

There has been a greater understanding of established inflammatory conditions of the oesophagus and the stomach in recent years. There have also been descriptions of newer entities. Some of these entities are novel however many are related to newer treatment modalities and medications including biologicals and immunotherapy. Diagnosis of drug-induced injury in the upper gastrointestinal tract is difficult. Some compounds are associated with characteristic patterns of injury, however, many are not. Patterns of injury generally are not specific and mimic other gastrointestinal conditions. In addition, these therapies and other immunosuppressive conditions predispose to infection. One proposed new entity is lymphocytic oesophagitis. There is recent evidence that this histological pattern is associated with primary oesophageal motility disorders. Lymphocytic and collagenous gastritis may be part of a pan-enteric inflammatory disorder, while isolated forms have also been seen secondary to Olmesartan therapy. While eosinophilic oesophagitis is now widely recognised both clinically and pathologically, eosinophilic gastritis remains rare and enigmatic. Finally, Crohn's disease affects the oesophagus and stomach not uncommonly and is often overlooked.

◆ Appendiceal Neoplasia and Pseudomyxoma Peritonei

Ⓟ NJ Carr

Peritoneal Malignancy Institute, Basingstoke, UK

Mucinous appendiceal neoplasms are not uncommon. Correct diagnosis is important because some are managed with prolonged follow-up or radical surgery. Mucinous appendiceal neoplasms may be low grade or high grade, and are distinguished from mucinous adenocarcinoma by a lack of infiltrative invasion. The latest edition of the WHO Classification will be based on the consensus classification of the Peritoneal Surface Oncology Group International (PSOGI). A fairly common problem is the differential diagnosis of mucinous neoplasm from ruptured appendiceal diverticulum. Both can show crypt disarray, epithelial serration and extravasation of mucin outside the appendix. Favouring a diverticulum are hyperplastic features confined to the luminal portion of the mucosa, preservation of essential mucosal architecture and intramucosal neuromas. Pseudomyxoma peritonei is a rare and distinctive syndrome that is due to an appendiceal primary in the vast majority of cases, although occasionally other primary sites may be responsible. The presence of signet ring cells confers a worse prognosis, and such lesions are now identified separately. When a low grade ovarian mucinous neoplasm is associated with true pseudomyxoma peritonei the appendix is usually the primary site. The exception is a low grade mucinous neoplasm arising in a mature teratoma of the ovary; such lesions not only behave identically to appendiceal lesions but also show the same genetic abnormalities. There are histological features that can point to an ovarian mucinous tumour being metastatic rather than primary, and SATB2 expression is a promising new marker in this respect. Goblet cell carcinoid tumours are an unusual type of neoplasm that almost invariably arise in the appendix. The term "carcinoid" is confusing in this context because they are actually a type of adenocarcinoma and are not related to neuroendocrine tumours. The latest edition of the WHO Classification will eliminate "carcinoid" from its nomenclature.

◆ Tumour Testing and Developments in the Understanding of Carcinogenesis in Lynch Syndrome

Ⓟ IM Frayling

Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK

Recent work is showing that there is more than one way that colorectal cancers develop in Lynch syndrome (LS), and that the immune system plays a critical role in determining which pathway an individual cancer follows. Moreover, this may help to explain the differing propensities to cancer associated with inherited pathogenic variants in the different genes. This has important implications for the tumour tests already carried out, and opens up other possible tests that may be worthwhile. It is now realised that there are at least three pathways to a large bowel cancer in LS. Firstly, LS patients can and do develop sporadic adenomas, initiated by mutations in the *APC* gene, like anyone in the general population. These then acquire a sporadic somatic mutation in the normal allele of the respective MMR gene, to become a cancer with DNA mismatch repair deficiency (dMMR). Secondly, one of the ~10,000 dMMR crypts that a LS patient has in their colorectal mucosa undergoes a mutation in beta-catenin, resulting in a flat intra-mucosal lesion which is not polypoid, but is immediately invasive. Thirdly, some of these lesions acquire secondary *APC* mutations, thence to become adenomatous, polypoid and eventually a cancer. Because dMMR leads to the production of highly antigenic frameshift peptides (FSP), LS patients have increasing titres of antibodies to such antigens and also have strong specific T-cell responses. This keeps their propensity to develop tumours in check, to some extent, but also reveals a variety of mechanisms of immune escape, relevant clinically. It is important to note that there is no evidence that tumours in LS develop any quicker than any others, and that the raised mutation rate in LS (and other) tumours is not in itself a driver in carcinogenesis. Rather, it is derangement of cell-cycle control and apoptosis by mutations in MMR proteins that is the driver, with implications for the functional tests used to interpret possibly pathogenic MMR gene variants.

◆ TNM8 and RCPath 2017: Updates and Controversies

Ⓟ NP West

University of Leeds, Leeds, UK

The eighth edition of TNM staging was published in 2016 and has been in routine use since the 1st January 2018 in the UK. Prior to TNM8 going live, the Royal College of Pathologists published the fourth edition of the guidelines for reporting colorectal cancer to ensure consistency with TNM8. The serial changes in TNM staging, often based on limited evidence, have caused significant controversy internationally over recent years. Subsequently several countries, including the UK, resisted the change from TNM5 to TNM6 and TNM7. Reporting requirements have therefore markedly changed for UK pathologists from a staging system that has been in routine use since 1997. This talk will summarise the reasons why the UK has now finally opted to adopt the latest guidance, the changes introduced in TNM8 when compared to TNM5 for colorectal cancer reporting, and will highlight where UK practice continues to deviate from the UICC guidance. It will also attempt to give practical guidance in difficult areas such as tumour deposits and regression grading, and show some examples of cases where staging is changed depending on the version of TNM used.

◆ Industrial Centre for AI Research in Digital Diagnostics (iCAIRD)

Ⓟ G Bryson

Queen Elizabeth University Hospital, Glasgow, UK

The industrial Centre for Artificial Intelligence in Digital Diagnostics (iCAIRD) is an Innovate UK funded innovation centre based in Glasgow but with links and academic and industry partners across Scotland and the UK. The centre includes digital pathology (with main partner Philips) and radiology (main partner Canon). We are building on an existing integrated programme of pathology digitisation across NHS Scotland, academic expertise in image analysis and AI in digital pathology, and strong industrial partnerships.
For digital pathology, there are three main focuses:
1. The first step required for development and adoption of AI in pathology is the widespread implementation of primary diagnostic digital pathology. We will fully digitise the Pathology Department at the Queen Elizabeth University Hospital – currently the largest in the UK (over 110,000 requests p.a.; 47 Consultant Pathologists). As part of this deployment, we are co-developing and implementing a reporting solution to enable a distributed digital pathology deployment.
2. The current cellular pathology workforce crisis requires new efficient ways of working. As an exemplar project, we aim to increase capacity by developing AI algorithms to assist in the efficient reporting of endometrial and cervical biopsies.
3. We will capture high volumes of images and linked data in a EPCC/HDRUK database to act as a resource for the development of future precision digital diagnostics.

◆ The Use of Light Sheet Microscopy to Develop Novel Approaches to Define Vascular Structure in Health and Disease

Ⓟ KJ Griffin

University of Leeds, Leeds, UK

Purpose of the study: This presentation will introduce light sheet microscopy and will discuss the associated tissue processing protocols and imaging workflow. This will be approached in the context of using light sheet microscopy to develop novel approaches to define vascular networks in health and disease (Pathological Society Career Development Fellowship).
Methods: Tissue clearing; immunolabelling; light sheet imaging; post-processing workflows.
Summary of results: Diabetes mellitus (DM) is a global health burden which will affect ~500 million people by 2035. In DM, healthy tissue (be that brain, myocardium or skeletal muscle) is lost because of a failure of adequate tissue perfusion. Our group studies the factors influencing neovascularisation in a murine model of critical limb ischaemia (CLI). The ability to quantitatively analyse vessel development within tissue samples is a crucial part of this work, which, to date, has only been possible in 2-dimensions. Moreover, when studying murine and human skeletal muscle vasculature, it is routine practice to rely on immunofluorescent labelling of cryosections, imaged with confocal microscopy. These approaches focus on small tissue regions, meaning we could neglect important spatial differences in pathology, and fail to appreciate the interplay between the vascular, nervous, and immune systems in recovery from ischaemia.
Conclusions: Using light sheet microscopy of optically cleared murine tissues we create topological 3-dimensional maps of our pre-clinical samples, thus offering a greater scope to understand and quantify vascular remodelling. Once optimised, we will extend these developments to the study of limb ischaemia and ulceration in patients with DM. In the future these technologies could potentially be translated into clinical practice, for example in the assessment of the micro-circulation to individualise patient treatment in CLI and better predict functional outcome following lower limb revascularisation.

◆ Changing Landscape in the Management of Advanced Bladder Cancer

Ⓟ SA Hussain

University of Sheffield, Weston Park Cancer Centre, Sheffield, UK

Several new drugs have emerged in the management of advanced metastatic bladder cancer that have shown exciting results in randomised clinical trials. The clinical trials of immune check point inhibitors in advanced bladder cancer has shown promising results. Proportion of patients with metastatic bladder cancer treated with immune check point inhibitors in second line setting and achieving long term durable responses has increased. Their use in first line setting and neo-adjuvant and adjuvant settings are being tried in clinical trials. Improvements in our understanding of tumour biology of bladder cancer and further sub classification of bladder cancer is helping to provide more insights into choices of treatments and new drugs are being developed for targeted therapies in bladder cancer. Clinical trials in second line/third line have also shown promise. Trial of Docetaxel plus Ramicurimab versus Docetaxel and Placebo met its primary end point with imporvement in progression free survival. Role of Erdafitinib in biomarker positive patients has recently received FDA approval and is the first biomarker driven drug to get approval in advanced bladder cancer. Amongst the myriad of promising drugs there will undoubtedly be some that would fail to meet current hopes, but we can be optimistic that using this approach of robust molecular profiling and preselecting patients within clinical trials with matched treatments, more and more drugs will find a useful place in keeping advanced cancer at bay for longer than can be achieved at present. This will also help to move some of these exciting targeted therapies to earlier stages of cancer as single agents or in combination with established therapies within clinical trials.

◆ The Management of Gynaecological Cancers in Lynch Syndrome: The Manchester International Consensus Meeting

Ⓟ EJ Crosbie; NAJ Ryan; Manchester International Consensus Group; DG Evans

University of Manchester, Manchester, UK

Purpose: There are no internationally agreed clinical guidelines as to how best to manage the risk, prevention and treatment of gynaecological cancers in women with Lynch Syndrome. The Manchester International Consensus Group was convened in April 2017 to develop clear and comprehensive clinical guidance regarding the management of the gynaecological sequelae of Lynch Syndrome based on existing evidence and expert opinion from medical professionals and patients.
Methods: Stakeholders from Europe and North America worked together over a two-day workshop to achieve consensus on best practice. Stakeholders included patients, patient support groups, gynaecologists, clinical geneticists, medical oncologists, colorectal surgeons, gastroenterologists, histopathologists, genetic pathologists, health economists, epidemiologists, gynaecology nurse specialists and genetic counsellors.
Results: Guidance was developed in four key areas: (1) whether women with gynaecological cancer should be screened for Lynch Syndrome and (2) how this should be done; (3) whether gynaecological surveillance was of value for women with Lynch Syndrome; and (4) what preventive measures should be recommended for women with Lynch Syndrome to reduce their gynaecological cancer risk.
Conclusion: The Manchester International Consensus Guideline provides comprehensive clinical guidance that can be referenced by both patients and clinicians so that women with Lynch Syndrome can expect and receive appropriate standards of care.

◆ Molecular DCIS Update

Ⓟ EJ Sawyer

Kings College London, London, UK

Existing methods for accurately predicting the behaviour of DCIS are poor and the management of patients with established DCIS is very varied, including complete surgical excision with or without additional radiotherapy and/or hormone therapy.
The development of biomarkers that can predict recurrence/progression of DCIS would dramatically impact on the clinical care of women with DCIS. There is concern regarding over-treatment and this is reflected in new clinical trials of DCIS offering observation alone following biopsy for low and intermediate grade DCIS (LORIS trial in UK and LORD trial in the Netherlands). However, for some women omitting radiotherapy after BCS is associated with a high risk of development of invasive disease and for observation-only to be a viable treatment option, it is imperative that biomarkers are identified in order to avoid under treatment of those most at risk. The Sloane Project is a UK-wide prospective audit of screen-detected, non-invasive and atypical hyperplasias of the breast. The Project started in 2003 and now has data on 10,500 cases of DCIS diagnosed in the UK between 1/4/03 – 31/3/12. From this cohort, we have a identified a series of DCIS cases that have developed an ipsilateral recurrence after breast-conserving surgery and a series of DCIS that have not recurred. Molecular analyses of the primary DCIS tissue has been performed including copy number analysis, targeted sequencing and RNA seq in collaboration with the DCIS_PRECISION team. The preliminary analysis of this data will be presented.

◆ Tricky Topics for Trainees on Soft Tissue Pathology

Ⓟ K Thway

Royal Marsden Hospital, London, UK

Soft tissue tumors are a group of rare childhood and adult neoplasms with differentiation towards mesenchymal tissue, which can originate anywhere in the body. There are over 150 sub-types of benign and malignant soft tissue tumors. They are pathologically diverse and complex, but often show highly overlapping morphological and immunophenotypic characteristics, and benign soft tissue tumors can be close morphological mimics of highly aggressive malignant neoplasms. Soft tissue tumors frequently exhibit similar clinical and radiologic presentations, and histology therefore remains the cornerstone of diagnosis. Due to their rarity and the often infrequent exposure to soft tissue tumors outside of tertiary centers, soft tissue pathology can present a real challenge in training. This session will focus on some key diagnostic challenges in soft tissue pathology, and aims to demonstrate a basic, safe approach to dealing with soft tissue tumors, correlating clinical features with morphology, immunohistochemistry and molecular diagnostic approaches.

The following Plenary, Oral and Poster abstracts have been subjected to peer review

Digital Pathology for Primary Diagnosis of Screen-Detected Breast Lesions: A Review of the Literature and Experience from Four Centres

Ⓟ BJ Williams[1]; J Besusparis[2]; D Clark[3]; D Snead[4]; A Hanby[1]; R Millican-Slater[1]; E Verghese[1]; A Nijhawan[1]; D Treanor[1]; E Rakha[5]

[1]Leeds Teaching Hospitals NHS Trust, Leeds, UK; [2]National Center of Pathology, Vilnius, Lithuania; [3]United Lincolnshire Hospitals NHS Trust, Grantham, UK; [4]University Hospitals Coventry and Warwickshire, Coventry, UK; [5]Nottingham University Hospitals NHS Trust, Nottingham, UK

Purpose of the study: The rate of deployment of digital pathology(DP) systems for primary diagnosis in the UK is accelerating, with departments seeking to capitalise on efficiency, workflow and quality improvements. The innate flexibility and resilience of digital slides versus standard glass slides could be of great benefit in NHS the breast cancer screening programme (NHSBSP). This study aims to address the safety and benefits of DP to the NHSBSP.
Methods: A literature review was performed to identify studies describing the use of DP for primary diagnosis of breast lesions and pertinent to the NHSBSP. In addition, data from 4 sites, including unpublished experimental and validation data were subjected to detailed concordance:discordance analysis making this the most comprehensive synthesis of digital breast cancer diagnostic data to date.
Summary of results: Detailed concordance analysis of experimental data from 2 histopathology departments reveals complete clinical concordance rates for breast biopsies of 96% (216/225) and 99.6% (249/250). Data from direct comparison validation studies in 2 histopathology departments, utilizing the protocol recommended by Royal College of Pathologists found complete clinical concordance rates for breast histology cases of 99.4% (180/181) and 99.0% (887/896). Discordances encountered in the studies most frequently concerned minor discrepancies in grading attributable to identification of weddelite calcification and differences in mitotic count scoring that is comparable to the published intra-observer concordance figures.
Conclusions: The experience of 4 histopathology laboratories, in addition to our review of pre-existing literature suggests that DP is safe for the primary diagnosis of breast histology specimens, and does not increase the risk of misclassification of breast biopsies.

Detecting Driver Mutations and Potential Future Drivers in Early Stage Lung Cancer and Investigating the Potential to Expand this to a Sputum Screening Programme

Ⓟ HM Wood[1]; E Verghese[2]; H Slaney[1]; S Dixon[2]; R Milton[2]; M Kefaloyannis[2]; P Tcherveniakov[2]

[1]University of Leeds, Leeds, UK; [2]Leeds Teaching Hospitals NHS Trust, Leeds, UK

Purpose of the study: As personalised medicine becomes more prevalent, it is becoming increasingly important to identify the molecular drivers in cancer. However, personalised treatment can lead to new drivers emerging which are resistant to the original treatment, so knowledge of mutations in sub-populations of cells is also important. In addition, using the detection of mutations in non-tumour samples as a screening tool, so called liquid biopsies, has become a growth area of research.
We sought to use the new high-sensitivity sequencing kits to detect clonal and sub-clonal mutations in a cohort of early stage lung cancer patients. We then attempted to find the same mutations in sputum samples collected before surgery, in a mimic of a potential screening setting.
Methods: Tumour resections and sputum samples were collected from eight early stage lung squamous cell carcinoma patients. DNA was extracted, and libraries prepared with the Agilent SureSelect HS kit, using a lung cancer panel. Samples were sequenced on an Illumina HiSeq, and mutations called using bespoke high-sensitivity pipelines.
Summary of results: All patients showed at least one high cellular frequency deleterious mutation in a known lung cancer driver gene, such as *TP53* and *KRAS*. All of them also showed potential drivers at lower frequencies. None of the driver mutations were detected in the sputum samples.
Conclusions: Sequencing kits designed to have high sensitivity to low cellular frequency mutations can be a useful tool when stratifying patients in a personalised medicine setting, allowing the detection of the likely main driver mutations, and potential drivers that may emerge under the selective influence of a treatment regime. We found no evidence that sputum samples are a useful screening tool using this technology, maybe because few tumour cells or tumour DNA molecules are found in sputum. Other forms of liquid biopsy are likely to be more effective screening techniques.

Invasive Breadth as a Novel Prognostic Marker for Malignant Melanoma

Ⓟ A Khanna[1]; M Bamford[2]; GS Saldanha[1]

[1]University of Leicester, Leicester, UK; [2]University Hospitals of Leicester, Leicester, UK

Purpose of the study: In the UK Melanoma is the fifth commonest cancer. Histological markers are crucial to staging. The AJCC cancer staging manual 8th edition regards tumour thickness as the strongest predictor for melanoma specific survival (MSS). Histological breadth of invasion has never been investigated. We aim to investigate the impact of histological invasive breadth on survival, using 1004 samples. We hypothesise that breadth is a valid prognostic feature.
Methods: The invasive breadth was defined as the horizontal distance between the lateral most invasive components of a melanoma. Measurements were carried out using hematoxylin and eosin stained slides and standard light microscopy. 1,209 patients with primary invasive melanomas, diagnosed at the University Hospitals of Leicester (UHL) between 2004-2012, were eligible. 1,004 patients were included in the study. Data was acquired through original pathology reports and UHL patient databases. Bivariate analysis of breadth and other prognostic features alongside and univariate / multivariate survival analysis with MSS as primary outcome were carried out using R.
Results: Bivariate analysis showed strong association between breadth and other histological variables such as Breslow thickness (BT) and mitoses. Multivariate analysis showed that breadth was strongly associated with MSS, p value <0.001, adjusted hazard ratio (HR) 1.11(CI 1.07-1.15), however adjusted BT HR was not significant. BT was significant in a multivariate analysis only when breadth was not in the model. Analysis showed that an interaction effect was present between breadth and BT. To explore this further the data set was divided into 2 groups of BT≤ 2mm and BT > 2mm. This showed that breadth was significantly associated with MSS in thicker melanomas, but not thin and vice versa for BT.
Conclusion: This study showed that breadth was strongly associated with MSS even after adjustment and may predict MSS better than BT. Further research is necessary in order to validate our findings.

Use of T Cell-Specific RNA In Situ Hybridisation as a Novel Test to Distinguish Malignant (Lymphomatous) and Benign (Inflammatory) T Cell Infiltrates

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Background: Differentiating benign from malignant (lymphoma/leukaemia) lymphocytic infiltrates is an important and common clinicopathological. For T cell infiltrates, there is no equivalent of kappa/lambda immunoglobulin light chain staining, meaning that expensive and time-consuming T cell receptor gene rearrangement PCR studies are needed. We identified two T cell-specific, mutually exclusively expressed, RNA sequences (TRBC1 and TRBC2), corresponding to the two, alternatively employed, T-cell receptor beta constant regions.
Methodology: We analysed the TRBC1/2 gene segments using standard bioinformatic tools, undertook Q-PCR to investigate relative expression levels and developed single and duplex TRBC1/TRC2 segment-specific probes for chromogenic in situ hybridisation (CISH), which we validated on FFPE sections of T-cell lymphoma/ leukaemia lines, T-cell lymphoma (n=100) and corresponding benign tissue (n=100).
Results: The coding regions of TRBC1/2 are very similar at amino acid level, making development of highly specific TRBC1/2 monoclonal antibodies difficult. However, the 3' untranslated regions differ substantially and Q-PCR demonstrated the TRBC1: TRBC2 transcript ratio in peripheral blood mononuclear cells to be very close to 1:1. This was confirmed by single and duplex TRBC1/2 CISH staining of benign T-cell infiltrates. CISH staining of T-cell lymphoma/ leukaemia lines and FFPE sections of T-cell lymphoma demonstrated clear TRBC1/2 restriction (monotypia for TCR), correlating with Q-PCR results. Double (duplex) CISH staining can distinguish between benign (inflammatory) and malignant (lymphomatous) lymphocyte populations in a wide range of tissues.
Conclusion: This is the basis of a novel diagnostic test for T-cell lymphoma, applicable to FFPE sections, that might replace PCR-based clonality studies in the majority of cases, transforming the routine assessment of T cell infiltrates, analogously to kappa/lambda staining for B-cells.
This abstract has previously presented at the European Hematology Association 24th Congress, Amsterdam: 13–16 June 2019.

Prognostic Value of Spatial Interactions of PD-L1$^+$ Cells in Oropharyngeal Squamous Cell Carcinoma: The Pattern Matters

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Purpose of the study: Immune checkpoint inhibitors, particularly those targeting the PD-1/ PD-L1 pathway of immune-escape, are revolutionising treatment in Oropharyngeal Squamous Cell Carcinoma (OPSCC). There is a need for biomarkers to identify high-risk patients with immuno-incompetent tumours. Observing patterns between cell phenotypes is a promising avenue for biomarker development and to improve our understanding of the tumour micro-environment, the inherent complexity of which is often lost in single-plex immunohistochemical analysis. We aimed to quantify patterns between PD1$^+$ or CD8$^+$ and PD-L1$^+$ cells and test their prognostic significance in OPSCC.
Methods: Diagnostic biopsies from 72 OPSCC patients were stained using multiplex immunofluorescence for CD8, PD1, PD-L1 and CD68. Multispectral scanning and spectral unmixing identified cells positive for each of the markers. The Hypothesised Interaction Distribution (HID) method quantified patterns by the spatial proximity between cells positive for PD1 or CD8 and PD-L1. Patterns were correlated with overall survival.
Results: High frequencies of PD1$^+$ and PD-L1$^+$ (HR 2.64, p=0.042) cells occurring within 30 µm of each other were associated with a poor outcome in patients with HPV negative (n=31) OPSCC. The same effect was observed for co-occurring CD8$^+$ and PD-L1$^+$ cells (HR 2.95, p=0.025).
Conclusions: The HID method can automatically quantify spatial interactions and identify poor-prognosis OPSCC patients. Frequent co-occurrence of PD-1$^+$ or CD8$^+$ and PD-L1$^+$ cells should indicate immune escape through the PD-1/PD-L1 pathway. Future work should validate these finding in larger cohorts and test other interactions between lymphocyte subsets.

A Quantitative Evolutionary Approach Utilising High Resolution Chromosomal Copy Number Analysis Accurately Stratifies Patients with Ulcerative Colitis and Low Grade Dysplasia by Future Colorectal Cancer Risk

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Introduction: Low grade dysplasia (LGD) in ulcerative colitis (UC) demonstrates a variable risk of progression to colorectal cancer (CRC). Chromosomal copy number alterations (CNAs) are known to occur in UC epithelium. The correlation between LGD CNA burden and future CRC risk is unknown. Shallow whole genome sequencing is a novel, cost-effective technique for high resolution CNA analysis in formalin-fixed, paraffin-embedded tissue.
Methods: We analysed 34 LGD lesions from 22 'progressor' patients who subsequently developed HGD/CRC a median 427 days later (IQR 218-907), and 49 LGD lesions from 45 matched 'non-progressor' patients who remained HGD/CRC-free for >5 years. Histological grading was confirmed by two blinded pathologists.
Results: Both maximal total CNA burden and number of CNA events are greater in LGD of progressor patients than in LGD of non-progressors ($p<0.001$). Specific CNA events occur at much higher frequencies in progressor LGD, including 4q loss, 5p gain, 17p loss and 17q loss (OR>20, $p_{adj}<0.01$). Multivariate analysis combining genetic, clinical and endoscopic data demonstrates CNA burden as the only significant risk factor for future CRC risk ($p<0.001$). Survival analysis of the combined 67 progressor and non-progressor patients demonstrates that those patients bearing LGD with the 25% greatest number CNA events and/or a CNA event on chromosome 17 are much more likely to develop CRC/HGD than the remaining patients (HR 14.8, $p<0.001$). ROC analysis combining clinical and genomic data allows for highly accurate CRC risk prediction, with an AUC of 0.92. Temporospatial phylogenetic analysis in 10 progressor patients with metachronous and/or synchronous neoplasia demonstrates evidence of both clonal expansion (multiple shared CNA events between lesions) and mosaicism (no shared events between lesions).
Conclusion: LGD demonstrates a surprising diversity in CNA burden; some LGD CNA profiles are indistinguishable from the CNA profile of the CRC which subsequently arises in that patient. Shallow whole-genome sequencing output can be used to accurately predict the future CRC risk of LGD.

Low Levels of Intra-Tumour Heterogeneity in Non-Muscle Invasive Bladder Cancer

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Purpose of the study: Non-muscle invasive bladder cancer (NMIBC) progresses to muscle invasive bladder cancer (MIBC) in up to 20% of cases with an associated increase in mortality and morbidity. Recently, intra-tumour heterogeneity (ITH) has been recognised as an important factor in treatment resistance and aggressive biological behaviour in numerous tumour types. However, ITH has not been investigated in NMIBC. We aimed to assess ITH of gene expression in NMIBC and its relationship to progression to muscle invasive disease.
Methods: We used multi-region sampling of formalin fixed paraffin embedded index NMIBC cases. Total RNA was extracted using the Qiagen Allprep kit. The Nanostring nCounter CancerPathways gene expression panel was used to give an overview of gene expression from networks important in cancer development. Intra-tumour heterogeneity was assessed by mean pairwise 1- Spearman's rank and mean pairwise Euclidean distance of gene expression between pairs of regions within each tumour. Overall tumour heterogeneity was compared between cases that progressed to MIBC and those that didn't.
Results: Ninety-six tumour regions were sampled from 23 patients, 10 of whom progressed to MIBC. Clinical and pathological characteristics were well matched between the two groups. Mean pairwise 1-Spearman's rank was 0.072 for non-progressors and 0.076 for progressors ($p=0.689$). Similarly, mean Euclidean distance was not significantly different between the two groups (20.89 vs. 19.58, $p=0.4777$). On hierarchical clustering, regions from each case clustered together.
Discussion: There are low levels of ITH in NMIBC and no significant difference in ITH is seen between cases that to progress to MIBC vs. those that remain localised. Future work could focus on the change in ITH over time and ITH in other molecular markers such as mutation profiles and miRNA expression.
This project was funded by a Cancer Research UK/Pathological Society Pre-Doctoral Research Bursary.

Histopathology at Autopsy: Why Bother?

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Background: Frequency of histopathological sampling and attitudes toward it varies nationwide. Inadequate sampling may limit the quality of autopsy reports.
Aim: To assess the value of histopathological sampling and identify barriers to taking it.
Method: Retrospective analysis of 141 autopsies undertaken at Manchester Royal Infirmary, a major teaching hospital, from January to June 2017. The gross and histological findings of the following were considered: brain, heart, kidney, liver, lung, and spleen. The number of pathological diagnoses recorded for each organ were categorised as concordant/refined, discordant, histology needed, and autolysed. Alterations to the recorded cause of death following histopathological sampling were categorised as direct, supportive, irrelevant and inconclusive. Additionally, seven consultant pathologists with post-mortem experience were interviewed. Each was asked six open questions in a semi-structured format. Transcripts were interpreted using thematic coding.
Results: The lung received the highest number (n= 320) and the spleen the lowest number (n= 35) of diagnoses. The organs most frequently requiring histology to reach a diagnosis were the kidney and lung: 52.8% and 28.8%, respectively. Histopathological sampling brought about an alteration to the cause of death in 45% of autopsy reports. In 9.3% of cases histology was not clearly relevant to the documented cause of death. Key barriers to sampling described by pathologists were time constraints and insufficient training.
Conclusion: This study found that histopathology has a major impact on determining an accurate cause of death. Inadequate exposure to post mortem histopathology during training can influence sampling habits as a consultant.

Deep Learning Analysis Identifies Lymphocytic Infiltration as a Prognostic Factor in Patients with Muscle-Invasive Bladder Cancer

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Purpose: Muscle-invasive bladder cancer (MIBC) is a highly aggressive disease and regardless of rigorous research the prognosis of MIBC patients has remained immutable during the last three decades. Patients with MIBC have a tendency towards a worse prognosis than patients with non muscle-invasive bladder cancer (NMIBC) and therefore there is a need to significantly ameliorate risk stratification. At present, bladder cancer evaluation depends principally on the clinical gold standard Tumour, Node, Metastasis (TNM) system for staging and patient prognosis. Although TNM staging is very accurate at predicting survival rates at the population level, it is less accurate at personalized prediction and does not adequately reflect the behaviour of the disease. However, features characterizing the immune contexture (IC) in the tumour microenvironment alongside TNM staging could improve the accuracy of patient prognosis. This study reports the identification of Tumour Budding, T-cells (CD3), cytotoxic T-cells (CD8) and programmed death ligand 1 (PD- L1) expression in MIBC patient samples. As a result, the combination of these features yields a superior prognostic signature in MIBC when compared with current TNM staging.
Methods: A computational imaging technology based on machine and deep learning was developed for the evaluation of Pan-cytokeratin, CD3, CD8 and PD-L1 markers across immunofluorescence (IF) labelled whole slide images from 100 MIBC patients, allowing for a comprehensive evaluation of the composition and distribution of distinct populations within the same tissue section.
Results: Our method achieved a more significant cohort stratification (Chisq=56.7, p-value=4.87x10-13) by utilizing the combination of CD8+ T cells infiltration and PD-L1 with TNM staging compared to TNM system alone (Chisq=47.7, p-value=4.45x10-11).
Conclusions: The computation of the IC by image analysis in combination with TNM staging correlates with an improved prognostic outcome.

Improving Adherence to NICE Guidelines for the Follow Up of NMIBC: Are We Over-Investigating?

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Bladder cancer is the tenth most common cancer in the UK, non-muscle invasive bladder cancer (NMIBC) accounts for 75% of these diagnoses. NMIBC a spectrum disease that is risk stratified based on histological type, size and foci. The high recurrence rate dictates a costly prolonged regular follow up period. NICE guidelines provide a surveillance scheme post-tumour resection that is based on risk of disease recurrence.
Method: Data from 100 patient's digital records over a 5 month period that had undergone treatment for a histologically confirmed NMIBC was assessed. Each case was risk categorised and the follow up data was then compared with the NICE guideline.
Results: Adherence to the guideline was poor, with a tendency to offer more cystoscopies than suggested by NICE. The low risk group was found to have the worse compliance, with the best compliance being in the high risk patient group. Overall, 77 unnecessary cystoscopies were carried out over the period.
Conclusion: The overall result of these deviations from the NICE guidance was a £17,479 increase in cost over the 5 months. The additional scopes also presented an increased potential for complications and patient distress from the procedure. The audit also incidentally highlighted the inconsistency of recording important details at the time of diagnosis, making risk stratification a challenge. Improved adherence to the guideline and standardized recording of histological type, size and foci would be cost effective, optimize resource allocation and reduce patient discomfort.

Pan-Sarcoma Molecular Fingerprints of Copy Number Change Reveal Distinct Tumour Biology

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Purpose of study: Sarcomas are a heterogenous group of mesenchymal tumours, with differing levels of genomic complexity, from simple-fusion driven tumours such as synovial sarcoma to highly copy number aberrant tumours such as undifferentiated sarcomas. We have previously identified molecular fingerprints in a dataset of 43 undifferentiated sarcomas; here we improve the identification method, and expand the identification to a pan-sarcoma dataset of copy number profiles.
Methods: Molecular fingerprints were extracted from sarcomas from the cancer genome atlas (TCGA) sarcoma dataset, and a series of published and unpublished sarcoma datasets. These included 112 undifferentiated sarcomas and myxofibrosarcomas, 71 uterine and soft-tissue leiomyosarcomas, 118 bone and soft-tissue osteosarcomas, 43 chondrosarcomas, 39 dedifferentiated liposarcomas, 5 malignant peripheral nerve sheath tumours, 6 synovial sarcomas and 10 low-grade sarcomas. Additionally, molecular fingerprints were probabilistically mapped to the human genome in all samples.
Summary of results: Previous molecular fingerprints associated with near-haploidisation and sequential genome doubling were recovered. The prevalence of genome doubling varies substantially between sarcoma subtypes. Chromothripsis amplification was identified on both a diploid and tetraploid background, predominantly observed in dedifferentiated liposarcoma. Fingerprint mapping correctly associated chromothripsis with known hotspots such as chromosome 12 leading to MDM2 amplification in dedifferentiated liposarcoma and parosteal osteosarcoma, however, other hotspots of chromothripsis remain unexplained.
Conclusions: Molecular fingerprints are a powerful tool to investigate the evolutionary history and copy number heterogeneity of sarcomas. The addition of mapping fingerprints allows for strong inference as to the copy number drivers of tumourigenesis in specific sarcoma subtypes, uncovering promising avenues for further research.

Deciphering Tumour Evolution in Neuroendocrine Lung Cancers

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Lung cancer is globally the biggest cause of cancer-related death, resulting in almost 1.6 million deaths per year [1,2]. The neuroendocrine family of tumours comprises 20-25% of all lung cancer diagnoses. Pulmonary neuroendocrine tumours lie on a spectrum of malignant behaviour, which range from the malignant, but relatively indolent, typical carcinoid tumours to small cell lung carcinoma- the most aggressive of the primary neuroendocrine lung tumours. Small cell lung carcinoma usually metastasises to lymph nodes and distant sites faster than other primary lung tumours, and has a bleak prognosis [3]. Although small cell carcinoma and other primary neuroendocrine lung tumours are considered part of the same family, the evolutionary relationship between these tumours is still poorly understood.

The TRACERx study (TRAcking non-small cell lung Cancer Evolution through therapy (Rx)) is a prospective cohort study across multiple UK centres which explores the genomic evolution of non-small cell lung cancers, identifying specific "driver mutations" which impact tumour behavioural phenotypes [4].

We apply the methodology and resources behind TRACERx to a cohort of primary bronchial neuroendocrine tumours, across a range of tumour grades. Multi-region sampling has been performed on each tumour whilst fresh, and whole-exome sequencing has been performed on each region of fresh frozen tumour. Utilising the established TRACERx bioinformatics pipeline, we explore copy number alterations and mutations identified in this group of tumours. We map evolutionary profiles of this tumour family and explore their interrelationships. We differentiate early clonal genetic changes from later sub-clonal changes. We correlate findings with histological subtype and clinical data to give a uniquely rounded perspective on the evolutionary behaviour of these tumours.

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The Role of Micro-RNAs 21, 200c, 204, 205 and 211 as Diagnostic Biomarkers of Benign, Dysplastic and Malignant Melanocytic Lesions

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Purpose of study: Overlapping histological features between benign and malignant lesions and lack of firm diagnostic criteria for malignancy result in high rates of inter-observer variation in the diagnosis of melanocytic lesions. We aimed to investigate the differential expression of five miRNAs (21, 200c, 204, 205 and 211) in benign naevi (n=42), dysplastic naevi (n=41), melanoma in situ (n=42) and melanoma (n=42), and evaluate their potential as diagnostic biomarkers of benign, dysplastic and malignant melanocytic lesions.
Methods: The expression profile of each miRNA was measured using real time PCR, with machine learning algorithms used to assess the diagnostic potential of differential miRNA expression. The spatial expression of miRNAs was demonstrated with chromogenic in situ hybridisation.
Summary of results: Real time PCR demonstrated differential miRNA expression profiles between benign naevi; dysplastic naevi and melanoma in situ; and invasive melanoma. Random forest accurately classified cases based on miRNA expression profiles with ROC curve analysis of 0.99 for malignant melanoma and greater than 0.9 for all other groups, indicating high accuracy of our panel of miRNAs as a diagnostic test. However, we also examine the significant impact of variable percentage of lesional cells, and of variable spatial expression patterns of miRNAs, on these PCR results. In situ hybridisation confirmed expression of miRNA-21 and 211 in melanocytes, while demonstrating expression of miRNA-205 primarily in keratinocytes, thus calling into question it's value as a biomarker of melanocytic lesions.
Conclusions: We have validated some miRNAs, including miRNA-21 and 211, as potential diagnostic biomarkers of benign, dysplastic and malignant melanocytic lesions. However, we also demonstrate the crucial importance of considering tissue morphology and spatial expression patterns when using molecular techniques for the discovery and validation of new biomarkers.

The Impact of Morbid Obesity and Weight Loss on the Immune Microenvironment of the Endometrium

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Background: Endometrial cancer (EC) has strongest association with obesity of all cancers; a 1.60-fold greater risk is conferred per 5kg/m2 increase in body mass index (BMI). Similarly, surgically induced weight loss reduces risk by up to 81%. It is proposed that this association is related to changes in the microenvironment. Although the immune microenvironment has been previously described in normal and neoplastic endometrium, no study has established if it is altered by weight loss.
Methods: Samples from a previous prospective study of morbidly obese patients undergoing bariatric surgery were utilised. 43 patients, ages 24-60, were included with three successive biopsies: at surgery, two-months and 12-months. Bloods were taken to collect further clinical data. Patients were predominantly pre-menopausal (37/43) with mean baseline BMI of 52.2 (SD=7.2). Multiplex immunofluorescence was used to simultaneously identify cells positive for markers CD8, CD68, CD3, FOXP3, PD1 and CD56. Primary outcomes were quantity of cells at each time point, repeated measures correlation with weight loss and with systemic inflammatory markers.
Results: Mean weight loss over 12-months was 29.2kg (SD=12.6). CD8+ (p=0.015, r=-0.32) cell density increased significantly over the 12-months. There was a significant reduction in inflammatory biomarkers CRP (p=1.38x10-5, r=0.58) and IL-6 (p=0.00082, r=0.46). CD3+ density negatively correlated with IL-6 levels (p=0.0028; r =-0.4896).
Conclusion: CD8+ cell density in the endometrium increased with surgical weight loss. CD3+ cell density rose, inverse to the fall in IL-6. This supports previous literature on EC immune microenvironments, suggesting these cells play a protective role in the endometrium. It may suggest the inflammatory state seen in obesity downregulates the immune system, as do tumours. These findings could have clinical impact in the development of prognostic biomarkers in EC or immunotherapy targets.

Serous Adenocarcinoma of the Endometrium (ESC): A Retrospective Review of Histological Features and Clinicopathological Correlation

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Purpose of the study: Endometrial serous carcinoma (ESC) is an aggressive neoplasm, due to its propensity for metastasis and recurrence. Our aim was to assess various histomorphological features of ESC and to see their clinicopathological correlation with disease-free survival (DFS) and overall survival (OS).
Methods: After Institutional Review Board approval, a total of 205 samples (belonging to 120 patients), diagnosed as ESC from Jan 2009 to Dec 2015, were retrieved and reviewed for various histology parameters. Electronic medical records, files were seen for clinical details and follow up. Receiver operating curves (ROC) were established for the diagnostic performance of depth of invasion (DOI), tumour-free distance (TFD) and myometrial invasion percentage (MI %). Overall survival (OS) and Disease-free survival (DFS) were generated by Kaplan–Meier curves and prognostic significance by Cox regression analysis.
Summary of results: The mean age at diagnosis was 61.8 years, mean tumour size was 4.01 cm. Pure serous carcinoma histology was seen in 67.5%; mixed histology in 32.5% cases. Endometrial intraepithelial carcinoma (EIC) was seen in 40% of cases.Myometrial invasion <1/2 was seen in 37/104 (35%)cases, more than/equal to half in 55/104 (52.8%)cases, while in 5/104 (4.2%) cases, no myometrial invasion was seen; however, 3 of these showed distant metastasis. P53 showed mutated type staining in 91% of cases. Follow-up data was available in 111 (92.5%) patients (median- 24.3 months).The mean DFS was 29.3 and mean OS was 31 months. Percentage myometrial invasion (40% cut off), absolute depth of invasion (6mm cut off) were found to be statistically significant ($p<0.05$) in the univariate and multivariate analysis for DFS. Tumour-free distance to serosa (</=7mm) also showed statistically significant association on univariate analysis with OS.
Conclusion: In ESC, calculating the percentage myometrial invasion and absolute depth of myometrial invasion will be more meaningful.

Expression Patterns of Immunohistochemical Markers p16 and HPV E4 on Biopsy Provide a Reproducible, Potentially Clinically Useful Classification of Cervical and Anal High-Grade Squamous Intraepithelial Lesions (SIL)

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Biopsy pathology usually decides treatment of cervical and anal potentially precancerous lesions, but is poorly reproducible, and does not clearly indicate a progressive lesion. Several studies have shown that grading p16/E4 immunohistochemical (IHC) staining provides a reproducible classification of high-grade (H-) SIL of both cervix and anus, the usual treatment threshold. In 318 women referred for colposcopy, we compared p16/E4 IHC grading of the worst cervical biopsy lesion with methylation of tumour suppressor genes FAM19A4/miR124-2 in cervical cytology (a marker for advanced transforming HSIL). We also examined in 119 women undergoing loop electro-diathermy excision (LEEP) for HSIL, the relation of E4/P16 IHC to the outcome of LEEP. E4 positive staining decreased with increasing SIL grade from 41% in LSIL to 3% in HSIL/CIN3. E4 positivity increased with grade of p16 when p16 expression was limited to the lower 2/3 of the epithelium (r=0.378), but fell with expression at higher levels in the epithelium. Loss of E4 expression was associated with methylation of FAM19A4/miR124-2 and (r=-0.177, p=0.010). 85% of women with ≥lower 2/3 p16 staining/E4-negative HSIL biopsies and 65% with limited p16 staining/E4-positive HSIL biopsies had ≥HSIL in the LEEP specimen (p=0.025). p16 expression in a cervical biopsy is related to viral production and transformation. Combined p16 expression in ≥2/3 of the epithelium and absent E4 relates to methylation, marks advanced HSIL, and indicates likely HSIL on a subsequent LEEP. Grading p16/E4 IHC provides a simple, reproducible, potentially clinically useful biopsy classification of SIL that relates to treatment and merits further clinical study in relation to the natural history and treatment of cervical (and anal) HSIL.

DNA Methylation in Amyotrophic Lateral Sclerosis

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Purpose of the study: ALS can be sporadic (sALS) or familial, with a number genes implicated including C9orf72 (C9ALS) and TARDBP. DNA methylation is an epigenetic mechanism whereby a methyl group is attached to a cytosine, usually resulting in gene expression repression. DNA methylation has been implicated in other neurodegenerative diseases, but little work has been conducted in ALS. We aim to elucidate the role of DNA methylation in motor neurone (MN) decline, without the interactions from other cell types, which may mask MN-specific DNA methylation changes.
Methods: Immunohistochemistry (IHC) was used to determine the pathology of 5-methylcytosine (5mC), TDP43 and 5-hydroxymethylcytosine (5hmC) within cervical spinal cord. From a subset of the same cohort, MNs were extracted from the anterior horn using laser capture microdissection (LCM). DNA was then extracted and analysed using the Illumina Methylation EPIC array.
Results: Immunohistochemistry revealed increased methylation in ALS, with C9ALS displaying the highest global methylation. Interestingly, methylation levels appeared to reduce in the minority of cells that showed loss of nuclear TDP43. Microarray data also showed significantly increased methylation for ALS cases when compared to controls, with PANTHER pathway analysis implicating many known neurodegenerative disease related pathways.
Conclusions: DNA methylation is a contributory factor in ALS, with our data suggesting hypermethylation in particular, is involved in ALS. Further study into the genes and promoters identified could help to elucidate biomarkers for ALS in the future.
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The Use of HOT_ARMS PCR in Liquid Biopsies for the Management of Patients With Colorectal Cancer

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HOT_ARMS PCR is an ultra-sensitive method for mutation detection. It is able to detect and quantify mutant DNA containing single nucleotide variants or indels down to a single copy. HOT_ARMS PCR can generate reliable signals for wild-type even when amplifying 100pg of circulating DNA. We sought to use HOT_ARMS PCR in liquid biopsies to provide data for patient management. 26 colorectal cancer patients were recruited to this study. Pre-/Post-surgery liquid biopsies and corresponding FFPE blocks were collected and analysed for KRAS exon 2 codons 12 and 13 mutations. Mutant allele frequencies of all samples were <3% and the DNA concentration range was 3.78-294ng/ml. 100% sensitivity was achieved by HOT_ARMS PCR (n=12) between FFPE tissue and pre-surgery cell-free-DNA. 7 samples presented surgical clearance and 5 samples had persistent mutant circulating tumour DNA signals in post-operative liquid biopsies. In contrast, nested full-COLD-PCR was deployed on the same cases and achieved 70% sensitivity even with a limit of detection of 0.75% mutant allele frequency. HOT_ARMS is a method of direct mutation detection and is far superior to mutation enrichment methods such as COLD-PCR for both detecting and quantifying mutations in liquid biopsies. Persistent mutant signals in post-operative samples indicate the utility of circulating tumour DNA for patient management. Ultra-sensitive mutation detection systems as characterized by this research are essential for early detection of treatment response. HOT_ARMS is a cheap, closed-tube test which can be universally applied to improve personalised cancer patient management.

An Assessment of EGFR Pathway Mutations and ALK Rearrangements in Lung Cancer by Next Generation Sequencing and Fluorescent In situ Hybridisation in a Regional NHS Genomic Testing Service

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Lung cancer is the most common cause of cancer death in the UK. For metastatic non small cell carcinoma (NSCC), targeted treatments against the epidermal growth factor receptor (EGFR) and rearranged anaplastic lymphoma kinase (ALK) may slow progression. Routine molecular testing of these targets is now recommended to determine eligibility. We aimed to analyse the molecular profiles of all cases tested through a large NHS regional genomic testing service over one year and correlate this with patient and sample factors. Pathology reports were analysed following lung cancer mutation testing for samples submitted between January and December 2018. All cases underwent next generation sequencing (NGS) of PCR products for hotspot mutations in EGFR and for other downstream genes including KRAS, NRAS and BRAF with an Illumina MiSeq. ALK rearrangement was determined by florescent in-situ hybridisation (FISH). Additional clinicopathological data were also collected. Data were collected for 280 patients; 131 male (47%) and 149 female (53%). Smoking history was recorded for 76% of patients (n=212) with a positive history in 92% (195/212).There were 263 tumours diagnosed as NSCC (94%). Where reported (n=277) sample type was 36% primary tumour (n=100) and 64% (n=177) metastasis. Sample type did not appear to influence the molecular result. Where tested, ALK was rearranged in 3% of cases (7/266). The mutation prevalences were 7% for EGFR (19/278), 31% for KRAS (85/275),1% for BRAF (4/275) and no NRAS mutations. The mutation and translocation prevalences were similar to that reported in the literature for EGFR (10-35%), BRAF (1%) and ALK (3-7%). The KRAS mutation rate was higher than expected (15-25%). The study population had a similar mutation frequency for EGFR and BRAF as well as for ALK rearrangement compared to the literature. This shows that routine molecular testing in advanced lung cancer can be successfully undertaken on a variety of sample types to help guide patient treatment.

Enhanced Glutamine Uptake Influence Composition of Immune Cells Infiltrates in Breast Cancer

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Purpose of the study: Cancer cells alter their metabolism in order to satisfy the demands of necessary energy and cellular building blocks. Glutamine availability for growth and progression of Breast Cancer(BC) is important in several BC subtypes. Immune evasion is an additional hallmark of cancer which plays a role in supporting tumour growth and progression.This study aimed to determine whether enhanced glutamine uptake in BC can derive the existence of specific subtypes of immune cells, including the subsequent impact on patient outcome.
Method: Solute Carriers(SLCs) involved in glutamine transport;SLC1A5,SLC7A5,SLC3A2, and immune cell subtypes;T-cell markers(CD3,CD8,FOXP3 andPD1),B-cell marker(CD20),histiocytic marker(CD68) and cancer-related immune marker(PDL1) were assessed using immunohistochemistry on TMA of a large BC cohort(n=803).Patients were stratified into accredited clusters based on SLCs expression, and correlated with the immune cell infiltrates, as well as investigating their associations with patient outcome.The effect of transient siRNA knockdown of SLC1A5 and SLC7A5 on PDL1 was evaluated in MDA-MB-231 breast cancer cell line.
Summary of results: The combined expression of all SLCs(High SLCs cluster) was significantly associated with tumour-related PDL1 and PD1+,CD20+,FOXP3+,and CD68+ immune cells($p<0.001$). In Triple Negative tumours, there were associations between High SLCs and PDL1 together with FOXP3+,CD68+ and PD1+ immune cells $p\leq 0.03$).The expression of SLCs and PDL1,FOXP3+,CD68+ cells was associated with poor survival while the expression with CD20+ cells was associated with better patient outcome ($p<0.001$).Knockdown of SLC7A5, but not SLC1A5, in TN cells significantly reduced the expression of PDL1.
Conclusion: This study provides pre-clinical evidence that altered glutamine pathways in BC, particularly TN tumours, appears to play a role in deriving specific subtypes of inflammatory infiltrates,which either support or counteract its progression.

Normothermic *Ex-Vivo* Perfusion of Human Lymph Nodes: A Feasibility Study

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Purpose of the Study: Precisely how much nodal disease necessitates an axillary lymph node (ALN) dissection in early breast cancer (BC) is contentious. Investigating which ALN metastases will progress, and refining how therapies might halt that process, is clinically important. However, modelling the complex ALN microenvironment is difficult.
Methods: Under appropriate ethical approval, we harvested ALNs from BC patients from whom it was clinically safe to do so. Firstly, ALNs from patients (n = 10) were perfused *ex-vivo* at 37°C for up to 24hrs. After confirming viability, targeted therapies were administered into perfusing ALNs to evaluate perfusion efficacy (n = 3).
Summary of Results: Controlled autologous testing showed that ALNs remain viable after 24 hours of *ex-vivo* perfusion: histology, proliferation and targeted gene expression did not change significantly over time for any perfused ALN compared with a control from time-point zero. During perfusion, although the acid-base balance of the perfused nodes remained stable, the flow rate through metastatic, but not reactive, ALNs increased significantly over time ($p<0.001$). Once viability was confirmed, targeted antibodies (Nivolumab and Trastuzumab) were administered into perfusing ALNs. These permeated the entire node, binding to their cognate receptors; Nivolumab even induced histological cancer cell death. Finally, Adrenalin injection into a perfusing ALN caused intra-nodal lymphovascular dilatation, proving that vasoactive drugs can be used to change the flow rate through ALNs.
Conclusions: We show that normothermic perfusion can keep human ALNs viable *ex-vivo* for hypothesis and intervention testing. This novel model might serve as a translational bridge, in which the efficacy of emerging personalised therapies and the crosstalk between tumour and immune cells could be investigated preclinically. Furthermore, the effects of changes in flow rate on the human ALN microenvironment could be evaluated in real-time.

Prognostic Significance of Isocitrate Dehydrogenase 2 (IDH2): A Biomarker Associated with Lymphovascular Invasion in Breast Cancer

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Background: Lymphovascular invasion (LVI) is associated with metastasis and is a prognostic factor in early-stage invasive breast cancer (BC). Through stringent bioinformatics analysis we identified Isocitrate Dehydrogenase 2 (IDH2) as one of the candidates gene associated with LVI positivity using multiple BC cohorts. This study aimed to evaluate the clinicopathological significance of IDH2 at transcriptomic and proteomic levels using large BC cohorts with long term follow-up.
Methods: IDH2 was probed at transcriptomic [using BC Gene miner, TCGA, and the METABRIC cohort] and proteomic level using immunohistochemistry in a large well-characterised BC cohort (n=859) prepared as tissue microarrays. Association with clinicopathological characteristics, and patient outcome were evaluated.
Results: In METABRIC and TCGA cohorts, overexpression of IDH2 mRNA expression was significantly associated with LVI-positivity (both $p<0.01$), high histological grade and HER2-positivity (all $p<0.05$). IDH2 mRNA expression showed significant positive correlation with IDH2 protein expression ($p=0.002$). At protein level, high expression of IDH2 was associated with LVI-positivity, high histological grade, high Nottingham Prognostic Index, HER2 positivity, large tumour size, and hormonal receptor negativity (all $p<0.01$). Increased IDH2 protein expression was significantly correlated with features of aggressive phenotype including Ki67, EGFR, and E-cadherin loss (all $p<0.05$). High expression of IDH2 mRNA and protein was associated with shorter 10 years of BC specific survival ($p=0.038$). In publicly available datasets using BC gene miner, up-regulation of IIDH2 mRNA was positively associated with poor outcome ($p=0.0002$).
Conclusion: This study confirmed the association of IDH2 expression with LVI status, tumour proliferation and metastasis related biomarkers; results warranting further functional validation and suggesting IDH2 as a potential therapeutic target in BC.

The Cell of Origin of Normal Human Hepatocytes has a Common Ancestor with Biliary Epithelium and Hepatocyte Clonal Expansions Arise from the Portal Tract and Drift to the Central Vein

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In high turnover epithelial tissues such as the intestine, homeostatic cellular replacement is achieved via a pool of stem cells. By contrast, turnover in the normal human liver is slow and the location and necessity of liver stem cells has been hotly debated. Partial hepatectomy has demonstrated that new hepatocytes can be generated from pre-existing hepatocytes, without the requirement of a stem cell population. However, lineage tracing in chronic injury models supports the involvement of liver stem/progenitors. Further complexity has arisen from the demonstration of both periportal and centrilobular neo-hepatocyte generation. This collective knowledge has largely amassed from rodent studies such that far less is known regarding the dynamics of human liver turnover, particularly during normal homeostasis. We have explored hepatocyte dynamics in normal human liver by utilising a combination of methylation of non-expressed genes and mitochondrial DNA (mtDNA) mutations as a molecular clock and clonal expansion respectively. Spatially, we show that clonal hepatocyte expansions are more commonly periportal than centrilobular. Furthermore, by laser-capture microdissection and mtDNA sequencing, we have demonstrated that hepatocytes and ductal epithelial cells have a common cell of origin. Using methylation status, an ancestral relationship was detected from periportal clonal hepatocytes, but not centrilobular. Lastly, using mtDNA next-generation sequencing, we have demonstrated that within clonal hepatocyte expansions, greater genetic diversity exists in centrilobular hepatocytes than those located in the same clonal patch periportally. This is the first study to show the stem cell of origin of normal human liver hepatocytes in the bile duct and stream towards the central vein. The periportal junction of biliary epithelium and hepatocytes has long been suspected as a location of liver stem/progenitors, thus this study demonstrates their involvement in liver homeostasis.

Liver Segment Sampling Using a Tru-Cut Biopsy Needle: Preliminary Data

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Aims: Sampling variation is a known issue in diagnostic liver pathology, limiting the histological interpretation of liver biopsy, and not entirely resolved by sampling protocols of explanted livers. We added to our routine diagnostic samples from explanted livers Tru-Cut biopsies from each liver segment to assess the feasibility of the technique and sampling variation.
Methods: A Tru-Cut needle biopsy was taken from each segment (I-VIII) of livers removed at transplantation. Each biopsy core was placed in a cassette labelled with the segment number. At embedding all segmental biopsies were laid sequentially into a single block, and oriented to have the segment 1 biopsy laid on the labelled side of the glass slides. Sampling variation was assessed on H&E and Sirius red stains using semi quantitative scores (Kleiner for steatohepatitis and Ishak for other aetiologies).
Results: This modified sampling protocol was applied to 17 cirrhotic livers from adult patients six (34%) cases of ALD, two (12%) each of NASH, HCV, PSC, PBC and hemochromatosis and one (6%) of HBV). A precise identification of segments was not possible in some instances (e.g. PSC) due to parenchymal remodelling. There was a variation in sample size and fragmentation. Biopsy surface area range was 9-21 mm^2. In terms of sampling variation, 45/46 (98%) biopsies from the steatohepatitis group showed stage 4 fibrosis, whereas 30 biopsies from chronic biliary disorders (PBC and PSC) showed fibrosis score varying from 3 to 6 in different segments, 6 being the predominant score in 18/32 (56%) biopsies. Eighteen biopsies from the viral hepatitis group showed variation in score ranging from 1-6, 6 being the predominant score in 11/18 (61%) biopsies, all of which belonged to the two cases of HCV cirrhosis.
Conclusion: Bench segmental Tru-Cut explant liver biopsy is feasible but limited by parenchymal remodelling, sample size variation and fragmentation. The underlying aetiology affects sampling variation.

Emerging Evidence of Cancer Stemcellness and Epithelial-Mesenchymal Transition in Hepatocellular Carcinoma Arising on Background of Hemochromatosis

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Purpose of study: Hereditary haemochromatosis (HH) is a risk factor for liver cirrhosis and hepatocellular carcinoma (HCC) with 8 to 33% of affected individuals developing HCC. It is thought that these patients fair worse than non-HH patients, however evidence is limited. HH patients also develop rare mixed subtypes, such as combined hepatocellular cholangiocarcinoma, suggesting that cancer stem cells (CSC) and epithelial-mesenchymal transition (EMT) contribute to the pathogenesis of HCC's in HH. This study aimed to identify whether characteristic features of EMT and CSC were observable in patients with HCC and whether such features were associated with clinical outcomes, clinicopathological features and preferentially concentrated within those patients with HCC on a background of HH (HH-HCC).
Methods: Explants or segmentectomies from 17 HH-HCC and 15 cases of non-HH aetiology HCC (nHH-HCC) were included. Survival rates, clinicopathological and demographic factors were compared. Presence of CSCs and EMT was assessed through immunohistochemical (IHC) staining with 7 antibody panels identifying each process.
Results: Men represent 88% of the HH-HCC and 86% of the nHH-HCC cohorts. HH-HCC had higher rates of combined tumour subtypes, tumour size and lymphovascular invasion, with none reaching statistical significance. HH-HCC patients displayed worse overall survival and decreased mean survival. CSC marker expression was increased in HH-HCC cases, with 56% positive for CSC markers (EpCAM and SALL4) compared to 17% of nHH-HCC. Morphological and IHC characteristics of EMT (loss of E-cadherin, CK18, and gain in vimentin, CD44) occurred with greater frequency in HH-HCC than nHH-HCC (37.5% vs 8.3%).
Conclusion: This study demonstrated that HCC arising on a background of HH has a worse prognosis compared to other aetiologies. CSCs and EMT were more prevalent in HH-HCC cases suggesting a pathogenic role for these processes in tumour progression in HH-HCC.

The Relationship Between DNA Mismatch Repair and Response to FOLFOX-based Pre-operative Chemotherapy in the International Phase III FOxTROT Trial

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FOxTROT is the first international phase III randomised clinical trial to evaluate the effectiveness of preoperative chemotherapy in locally advanced colon cancer. Biomarkers predictive of sensitivity to preoperative chemotherapy have not yet been identified. We investigated the relationship between mismatch repair (MMR) status and chemotherapy response. Patients were randomly assigned in a 2:1 ratio to pre- and post-operative chemotherapy comprising three 2-week cycles of FOLFOX then surgery followed by a further nine 2-week cycles, or to post-operative chemotherapy consisting of surgery followed by twelve 2-week cycles. H&E slides were collected for central pathological review in 904 out of 1052 cases (86%). Immunohistochemistry for MLH1, PMS2, MSH2 and MSH6 was performed in 794 patients (75%). Chemotherapy effectiveness was assessed blinded to trial arm using the AJCC 4 tiered-grading system.
Overall, 168 patients (21%) showed deficient MMR (dMMR). When blinded to trial arm, 7 patients showed complete regression, 13 near complete regression, 199 partial regression and 575 poor/no response. dMMR was associated with a significantly higher rate of poor/no response (96% vs. 66%, $p<0.0001$). Both groups had a low rate of complete or near complete regression (dMMR 1.8% vs. proficient MMR 2.7%, $p=0.494$). The trial arms will be unblinded in the near future. Whist the breakdown by trial arm is still awaited, analysis of the first 150 patients has shown that patients in the control arm are classed as poor/no response in 98% of cases. These provisional results therefore strongly indicate that patients with dMMR colon cancer are unlikely to benefit from FOLFOX-based preoperative chemotherapy. dMMR status should therefore be considered mandatory prior to considerations of chemotherapy to offer individualised treatment to patients.

Leukocyte-Associated Immunoglobulin-Like Receptor-1 Confers Poor Prognosis in Invasive Breast Carcinoma: Transcriptomic Driven Study

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Introduction: Leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1/CD305), is a transmembrane glycoprotein carrying immunoreceptor tyrosine-based inhibition motifs (ITIM) and it is reported to be overexpressed in high-grade tumours. Moreover, LAIR-1 plays a key regulatory role in immune cells function and extracellular matrix remodelling reflecting its role in tumour microenvironment homeostasis. The biological role of LAIR-1 in breast cancer (BC) has yet to be elucidated.
Methods: LAIR-1 expression was evaluated at transcriptome [using BC Gene miner, TCGA, and METABRIC cohort] and protein levels using immunohistochemistry in a large well-characterised BC cohort (n=569). In silico differential gene expression was used to evaluate LAIR-1 protein associated signalling pathways. Association with clinicopathological characteristics, immune cell subtype markers and patient outcome were evaluated.
Results: High LAIR-1 expression at the mRNA and protein levels were positively correlated with poor prognostic factors; high tumour grade, high Nottingham Prognostic Index, hormonal receptor negativity, P53, MMP2, MMP14 and MMP15 (all; $p<0.01$). Overexpression of LAIR-1 was also associated with T-cell markers (CD3, CD8, and FOXP3; $p=0.039$) and histiocytic marker (CD68; $p=0.022$). Multivariate analysis revealed that increased LAIR-1 protein is an independent risk factor for shorter BC-specific survival ($p=0.039$). Using BC gene miner, up-regulation of LAIR-1 mRNA was positively associated with shorter patient outcome ($p=0.025$). Inflammation mediated by chemokine and cytokine signalling pathway was the top predicted master regulator of LAIR-1 protein expression ($p=0.002$).
Conclusions: This study provides evidence for the prognostic value of LAIR-1 in invasive BC. Strong positive association with immune cell markers and LAIR-1 warrant further studies to assess them individually and in combination along with the immune checkpoint proteins.
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Immunogenomic Repertoire Profiling of Tumour-Infiltrating B Cells Reveals Sulfated-Glycosaminoglycans to be Major Functional Humoral Antigens in Human Malignancies

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Diffuse-type gastric cancer (DGC), among other gastric cancers (GCs), harbours "genomically stable" genotype with few neo-antigens; therefore, the efficacies of immune-checkpoint blockades alone may not be sufficient against DGCs. In order to understand precise molecular backgrounds of anti-tumour immunity in human DGC micro-environments, I aimed at immunogenetic profiling of tumour-infiltrating T and B cell repertoires for clinical DGCs. It was revealed that mature B cell immunity plays an important role in gastric cancers especially DGCs. With deeper focuses on the B cell repertoires, wide varieties of tumour-specific dominant immunoglobulins were discovered. Biochemical analysis of reconstructed IgGs of such dominant B cell clones showed that some of them exhibited auto-reactivities to abundant cellular proteins; however, it was of note that multiple of the rest of IgGs commonly recognized sulfated-glycosaminoglycans (sGAGs). More than 35% of the tumour-specific dominant IgGs found in human DGC tissues exhibited anti-sGAG nature. Intriguingly, those anti-sGAG human antibodies exhibited growth suppression against not only DGC cells but also various human cancers. Thus, sGAGs were revealed to be major functional B cell antigens in human tumour micro-environments and can be candidate targets of therapeutic antibodies for multiple of devastating human cancers.

Role of Intracellular Interleukin-1 Receptor Antagonist Type 1 in Oral Cancer and the Oral Senescence Program

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Purpose of study: The IL-1 receptor antagonist (IL-1RA) is a potent anti-inflammatory molecule, a major function of which is to inhibit IL-1. In endothelial cells, IL-1RA is also involved in the regulation of senescence and the senescence-associated secretory phenotype (SASP), two potent anti-tumour mechanisms that when de-regulated, can promote cancer development. IL-1RA is frequently downregulated in head and neck cancer (HNSC), but how this is related to the development of HNSC is unknown.
Methods: Using qPCR, western blot, confocal microscopy and immunohistochemistry, we analysed the expression of IL-1RA in a panel of different cell lines and biopsy samples. Transfection of icIL-1RA1 was done using a plasmid and knock down was done using CRISPR/Cas9. Senescence was assessed evaluating p16 and β-Galactosidase activity and SASP factors were analysed using western blot or ELISA.
Summary of results: Intracellular IL-1RA type 1 (icIL-1RA1) is downregulated in oral dysplasia (OD) and oral squamous cell carcinoma (OSCC). Transient re-expression of icIL-1RA1 in OSCC and OD cell lines showed limited or no effects on cell migration, cell proliferation and IL-6 and IL-8 secretion (important cytokines related to epithelial-to-mesenchymal transition ,angiogenesis and tumour growth). IL-1RA expression decreases significantly during normal and dysplastic keratinocyte senescence, which is accompanied by an increase in the expression of IL-1α, IL-1β and IL-6 and IL-8; two main markers of the SASP. Knock-down of icIL-1RA1 in pre-senescent NOK and OD cells caused a significant increase of IL-6 and IL-8.
Conclusions: IL-1RA is downregulated in OD and OSCC, but its phenotypic effects are not clear. IL-1RA downregulation during senescence is correlated with the increase of SASP factors, but this correlation still needs to be confirmed. Current work on CRISPR/Cas9 icIL-1RA1 knocked-out keratinocytes will help to understand icIL-1RA1 functions in senescence.

Optic Nerve Sarcoidosis Presenting as a 'Tumour' at the Optic Disc

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Background: Sarcoidosis is a chronic idiopathic granulomatous inflammatory disease that can affect any major organ system, primarily the lungs, and hence has remarkable heterogeneity in clinical presentation, findings and natural history. Between 25-80% of patients with systemic sarcoidosis will develop inflammatory eye disease and in approximately 20%, it may be the first clinical manifestation of the disease. The uveal tract is most commonly involved, although any segment of the eye and/or orbital structures can be affected. Herein we describe an unusual case of a subretinal 'tumour' at the optic disc.
Case report: A 61-year-old male presented with painful visual loss in the right eye. His previous history included Hodgkins lymphoma and mediastinal sarcoidosis. On examination, his right eye had no light perception, neovascular glaucoma, attenuated retinal vessels and a non-pigmented mass at the optic disc. The left eye was normal. The right was enucleated and sent for histology. Macroscopic examination revealed a whitish mass at the optic disc which histomorphologically showed fine anterior synechiae with focal angle closure and non-necrotising granulomatous inflammation at the optic nerve-head. Special stains for micro-organisms were negative. The appearances were those of optic nerve sarcoidosis. There was no evidence of malignancy.
Conclusion: Neurosarcoidosis is known as the 'great imitator' because it can cause non-specific clinical signs and a variety of symptoms mimicking many other conditions. It remains a challenging aspect of sarcoidosis, requiring prompt treatment to reverse eye damage and prevent permanent visual loss. A multidisciplinary approach is required to optimally manage ocular and systemic manifestations of sarcoidosis.

PDL1 and PDL2 Expression in Plasmablastic and Primary Effusion Lymphomas

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Purpose: Lymphoproliferative disorders have been shown to utilise PD-1/PD-L1 pathway to escape immune recognition. Limited data is available on PD-1 and its ligand expression in immune deficiency lymphomas; very small numbers of cases have been studied. PD-1/PD-L1 blocking agents may be a useful alternative to rituximab in treatment of these CD20 negative lymphomas.
Methods: Tissue microarrays were constructed using archived FFPE tissue for 17 previously diagnosed cases with adequate material (9 plasmablastic (PBL) and 8 primary effusion lymphomas (PEL)). All but one patient was tested to be positive for HIV; one other patient was on immunosuppressive treatment post-renal transplant. Standard immunohistochemistry (IHC) was utilised to determine the percentage of PD-L1 and/or PD-L2 positive tumour cells. RGB images of the IHC stained sections were imported into CellProfiler for analysis. Analysis on PD-1 is currently ongoing. Automated measurement of staining intensity for each cell was carried out by creating a binary mask using haematoxylin counterstain which was overlaid onto the DAB stained raw image.
Results: PD-L1 expression was noted in 8/9 PBLs. In three PBLs 33%, 5% and 3% cells showed strong expression of PD-L1; 1-31% cells showed weak/moderate intensity expression (mean: 13.2%). PD-L2 expression was noted in all 9 PBLs. In four PBLs 9%, 9%, 5% and 3% cells showed strong expression of PD-L2; 12-78% cells showed weak/moderate intensity expression (mean: 38.4%). PD-L1 expression was noted in 6/7 PELs. In one PEL 1% cells showed strong expression of PD-L1; 3-9% cells showed weak/moderate intensity expression (mean: 5.4%). PD-L2 expression was noted in 7/8 PELs. In three PELs 10%, 3% and 1% cells showed strong expression of PD-L2; 5-46% cells showed weak/moderate intensity expression (mean: 21%).
Conclusion: Our study demonstrated the potential benefit of immune checkpoint inhibitors in identification of a subset of patients whose tumours express PD-L1 and PD-L2.

Endocarditis and Sudden Cardiac Death

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Purpose of the study: Endocarditis is growing in incidence due to increased interventions, valve replacements and immunosuppression. It can be difficult to diagnose clinically and if left untreated can present as sudden cardiac death (SCD) with few or subtle preceding symptoms. True incidence of endocarditis related to SCD is unknown.
Methods: Retrospective analysis of our national database of 6000 cases of SCD, 1994-2018, for "endocarditis" as cause of death.
Summary of results: Of 21 cases (0.35% of total), 14(67%) were male and mean age was 32.6 ± 16.0 years. Post-mortem examination showed the aortic valve (AV) was affected in 12(57%), mitral in 5(24%), tricuspid in 3(14%) and pulmonary in 1(4.8%). Two (9.5%) were not valvular, both affecting graft repairs of the greater vessels. Five (24%) had coronary artery septic emboli and infarction. Twelve (57%) had an identifiable valve abnormality, prosthetic valve or previous valve operation, the most common being bicuspid AV (6/50%). Twelve (57%) had prior symptoms but only 7 (33%) had endocarditis diagnosed in life. Vegetations ranged from small, easily-missed irregularities to large and fungating.
Conclusion: This study highlights that although rare, endocarditis is an important cause of SCD in those with valvular disease and/or previous valve surgery. Preceding symptoms can be vague, and most individuals are not diagnosed during life. The absence of a pre-mortem diagnosis in almost 70% of our cohort highlights the need for thorough macroscopic pathological examination of the heart and cardiac valves. The gross appearance of vegetations can vary widely and lesions can be missed at autopsy.

Cancer-Associated Fibroblasts Expressing Proteins Required for Chemotaxis and Cell Migration are Prognostic in Non-Small Cell Lung Cancer

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Lung cancer is the leading-cause of cancer-related mortality with a 5-year survival in NSCLC of approximately 15%. After the success of immune checkpoint inhibitors, other cells in the tumour stroma, in particular fibroblasts, have become the focus of significant interest. In cancer, these cells become activated and are known as cancer-associated fibroblasts (CAFs). However, the prognostic significance and therapeutic targeting of CAFs has so far led to mixed results with studies suggesting CAFs are a heterogenous population. Identifying specific markers and pathways in CAFs which are prognostic in NSCLC might yield novel therapeutic targets as well as providing prognostic information. A systematic review of relevant articles was carried out via Ovid Medline. Data relating to survival were extracted from eligible studies. Pooled hazard ratios (HRs) were calculated for markers where they were examined in more than one study. In addition, identified markers were grouped together based on cellular process.
After exclusion of irrelevant titles and abstracts, 273/12120 articles were reviewed in full. This resulted in 49 eligible studies and 24 unique prognostic biomarkers. Meta-analyses showed no single marker had a significant effect on overall survival: CA IX (HR=1.40, p=0.32); α-SMA (HR=3.61, p=0.09); FAP (HR=1.36, p=0.62); and podoplanin (HR=1.72, p=0.30). However, analysis of cellular pathways showed markers involved in cell migration (cMET, α-SMA, podoplanin; HR=2.56, p=0.01) and chemotaxis (HGF, CXCL14; HR=2.63, p<0.01) were significant but not those required for glucose metabolism (IGF-2, GFAT-2; HR=4.71, p=0.25) or the response to hypoxia (MMP2, CA IX; HR=1.56, p=0.29). This study has identified that CAFs expressing proteins required for cell migration and chemotaxis are prognostic in NSCLC. The data also suggests that targeting these pathways in CAFs might yield novel therapeutic options rather than simple blockade of individual proteins.

Pulmonary Botryomycosis: A Mimicker of Malignancy

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We present a case series of pulmonary botryomycosis diagnosed in lung resections, and consider the histological features reported, with the aim of better defining this unusual but not uncommonly seen phenomenon.
Introduction: First described in 1870, botryomycosis is a bacterial pseudomycosis characterised histologically by the presence of mixed Gram positive and Gram negative non-filamentous bacterial colonies, often forming 'granules' within airways, with associated Splendore Hoeppli phenomenon, necrosis, fibrosis, multi-nucleate giant cells and granulation tissue formation. It is considered to be a chronic suppurative infection, arising in patients with impaired immunity.
Method: We carried out a freeword text search, 'botryomycosis', on our pathology system over a 20 year period. Confirmed actinomycosis cases were excluded. Clinical presentation, radiological appearance, microbiology results, histological features and special stains were recorded where available.
Results: 25 cases were identified, of which 16 are included, the remainder had a diagnosis of actinomycosis and therefore excluded. The patients comprise men (7) and women (9) with an average age of 53 years. Ten presentations were operated on for suspected malignancy, 6 with PET positive lung nodules. In two cases, the suspected pre-operative diagnosis was an aspergilloma. Two patients had coexistent low-grade neoplasms causing obstruction. Only 6 cases cultured organisms.
Conclusion: Botryomycosis is a rare infection causing mass-like lesions and should be considered in the differential diagnosis of suspected malignancy, especially when there is cavitation. The diagnosis is often made after surgical resection in this situation.

Multiplex Immunohistochemistry: The Next Generation of Molecular Pathology?

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Purpose of the study: Immunotherapy is a new paradigm in clinical oncology with durable tumour regression and stabilization of disease in advanced cancers, including non-small cell lung cancer (NSCLC). We recently reported on a comprehensive assessment of the PD-L1 diagnostic test in NSCLC. 1) A comparative validation of 22C3 (Dako) and SP263 (Ventana) PD-L1 clones. 2) A description of the PD-L1 reflex test in an accredited laboratory. 3) The role of digital pathology in the scoring of PD-L1. Importantly, we discussed the challenges of PD-L1 interpretation a) calculating the tumour cell denominator; b) peritumoural PD-L1 expression; c) calculation of positive tumour percentages at clinical thresholds; d) relevance of a 100 malignant cell rule. Here, we extend our investigation with the application of multiplexing to improve the accuracy of the PD-L1 diagnostic test.
Methods: Using OpalTM chemistry, on a Lecia Bond Rx automated staining platform, we optimised the multiplex method for sensitive and specific detection of PD-L1-SP263, cytokeratin-AE1 (CK) and CD68-SP1 to comparative routine DAB IHC. Spectral unmixing of each fluorescence channel and FFPE auto-fluorescence removal and was achieved with a Vectra Polaris scanner (40x) with image analysis/interpretation performed with inForm software.
Summary of results: Staining sequence optimisation achieved no discernible difference in antibody sensitivity or specificity following multiple antigen retrieval rounds. Comparison of DAB IHC and multiplex was highly concordant, as was the concordance of digital vs. manual assessment.
Conclusions: Phenotypic diagnostic analysis of PD-L1 is challenging. Making use of a validated multiplex (PD-L1, CK and CD68), we achieved specific visualisation of PD-L1 positive tumour cells. Visualisation of only positive epithelial cells was accomplished by CD68 subtraction. This study may represent a footprint for the validation of a lab-developed test for clinical molecular diagnostics.

Expression and Biological Significance of ADAM28 in Colorectal Adenocarcinoma

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While many colorectal cancers (CRCs) arise from adenomatous polyps through which we call "adenoma-carcinoma sequence", it has been estimated that up to 20% of CRCs likely evolve from an alternative pathway, so-called ''serrated pathway''. However, only 8–10% of CRCs display definitive serrated morphology at diagnosis. We previously examined expression of differentiation and molecular markers that are most likely to be involved in serrated tumour development by using 36 serrated CRCs and found CDX2 loss or BRAF mutations often together. We then generated a mouse model that can be induced concurrent biallelic inactivation of Cdx2 (Cdx2-/-) and expression of mutant BRAFV600E in adult mouse colon epithelium, and the Cdx2-/-/BrafV600E tumours showed a quite similar phenotype to that of CDX2-negative serrated CRCs. Through the validation of global gene expression profile including the mouse models and human CRCs, we focused on ADAM28 as a candidate because ADAM28 was significantly upregulated in Cdx2-/-/BrafV600E tumour and CRCs with low CDX2 expression and BRAFV600E mutation. We examined ADAM28 expression using 12 CRC cases with BRAFV600E mutation and 12 CRC cases with KRAS codon 12/13 mutations and found that ADAM28 was specifically upregulated in BRAF mutant CRC cases. Among 10 CRC cell lines, only HT-29, which has many phenotypic and genetic similarities to serrated CRC, showed a robust expression of membranous type ADAM28, and 9 of 10 CRC cells, except for RKO, showed weak to modest expression of active-form ADAM28. We present a novel transgenic model of human serrated CRC to highlight the suitability of centering on CDX2 loss and BRAFV600E in the pathogenesis. Through the validation of the gene expression profiles, we identified ADAM28 as a promising candidate of new biomarkers and therapeutic targets of CRCs with serrated morphology.

Expression of B7-H3 in Melanoma and its Effects on Patient Prognosis

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Purpose of the study: B7-H3 is an immune checkpoint molecule with a key role in regulating the immune response to cancer through the inhibition of T-cells. B7-H3 is overexpressed in a wide range of human cancers and is generally associated with poor prognosis and negative clinical outcomes. The aim of this project is to explore the relationship between B7-H3 expression in melanoma and patient prognosis with the hopes of identifying B7-H3 as an independent prognostic factor in melanoma. We will also test for association with numerous known clinicopathological factors of melanoma in order to gain a better understanding of the roles of B7-H3 in the progression of melanoma.
Methods: B7-H3 expression was detected by immunohistochemical assay in 328 and 405 cases of primary and metastatic melanoma respectively. Cases were categorised into positive and negative expression of B7-H3, based on the intensity and extent of staining observed. Correlation between B7-H3 expression and overall, recurrence-free and metastasis-free survival was analysed using Log-rank tests and Kaplan-Meier curves. Furthermore, correlation between B7-H3 expression and clinicopathological features was analysed using chi-squared and Fisher's exact tests.
Summary of results: B7-H3 was expressed in 42.6 and 47.4% of primary and metastatic cases, respectively. B7-H3 expression in both cohorts was not significantly associated with patient's survival. However, positive expression of B7-H3 was associated with adverse prognostic parameters in both primary and metastatic melanoma. Within the primary cohort, a significant association was identified with ulceration (p= 0.009) and a trend for positive correlation with microsatellites was found (p=0.061). In the metastatic cohort, B7-H3 expression was significantly associated with lymph node metastasis (p<0.001) and distant metastasis (p<0.001).
Conclusion: Overexpression of B7-H3 in melanoma tissue is significantly associated with adverse prognostic parameters.

The Role of Deep Learning in the Classification of Tumours of Fat

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Purpose of the study: Lipomas are common benign neoplasms of fat with an estimated incidence rate of 2.1 per 1000 people per year. Variation in lipoma histology and the large size of some lipomas require distinction from well differentiated liposarcomas which are malignant and rare and thus prove a histological and clinical challenge. The histological distinction is made by assessing nuclear and architectural features which requires review of multiple sections and the use of ancillary genetic testing. Tumours of fat can therefore present a considerable workload in the general pathology setting and often requires specialist review. Automated whole slide image (WSI) analysis could help address this problem however there are a lack of existing tools and the nature of the tissue presents a computational challenge.
Methods: We developed an iterative framework for quantitative analysis of WSIs using unsupervised clustering and deep convolutional neural networks (CNN) integrated with pathologist feedback. We used 55 liposarcoma and 32 lipoma H&E stained images as the training data with subsets held out for validation and testing. Two CNNs were employed, one using a low magnification (10x) tile based approach to capture architectural features where each WSI was split into an average of 50,000 tiles. We trained another CNN on high resolution spatially tracked nuclei (40x) to capture the nuclear features.
Results: The ability of the "nuclear" CNN to distinguish aberrant from normal yielded an 86.15% overall accuracy (F-score of 0.89) and 81.44% (F-score of 0.839) when using the "architecture" CNN alone. The accuracy for both CNNs was determined at a tile level rather than for the WSI.
Conclusions: Our preliminary results suggest that whole slide image analysis of fatty tumours is feasible but requires refinement. Ongoing work includes incorporating pathologist feedback, integrating both CNNs at the WSI level and then re-training the algorithm on a larger image dataset.

Synovial Chondromatosis, Synovial Chondrosarcoma and Soft Tissue Chondroma: Adding to the Group of Calcifying Tumours with a *FN1* Gene Rearrangement

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Synovial chondromatosis is a rare benign cartilaginous tumour in which a *FN1-ACVR2A* fusion has been described. Malignant transformation to synovial chondrosarcoma occurs in up to 10% of cases. Soft tissue chondromas share similar clinical and histological features with synovial chondromatosis, however, the former rarely recur and have never been reported to become malignant. Here we aim to determine if *FN1* and *ACVR2A* rearrangements represent recurrent alterations in benign and malignant synovial chondromatosis, and to identify recurrent genetic alterations in soft tissue chondromas. ***(Supported by a Path Soc Grant)***
Results: RNA sequencing of 4 synovial chondromatosis (1 malignant) revealed a *FN1-ACVR2A* fusion. RNAseq of 1 soft tissue chondroma revealed a *FN1-FGFR2* fusion, finding confirmed by FISH (*FN1* and *ACVR2A* break-apart probes). Rearrangements were detected in 33/58 synovial chondromatosis and 3/4 synovial chondrosarcomas. Rearrangements of *FGFR1 or FGFR2* were detected in 9/18 soft tissue chondromas. 3 of these 9 tumours revealed a *FN1-FGFR1* fusion, also found in phosphaturic mesenchymal tumours, a tumour which shares histological features with soft tissue chondromas but differ in that the former express FGF23 mRNA. This diagnosis was excluded by the absence of FGF23 expression by RNA in situ hybridisation (RNAscope) and qPCR.
FN1 and/or *AVCRA2* gene rearrangements do not distinguish between benign and malignant synovial chondromatosis. However, copy number alterations in *CDKN2A* were detected in 3/4 synovial chondrosarcomas. In conclusion, recurrent fusions involving *FN1* are observed in synovial chondromatosis, synovial chondrosarcoma and soft tissue chondromas. *ACVR2A* fusion appears to be restricted to synovial chondromatosis, and *FGFR1 or FGFR2* to soft tissue chondromas. Clinical assessment and/or RNAscope help distinguish between soft tissue chondromas and phosphaturic mesenchymal tumours. Genetic alterations in the remaining cases are yet to be identified.

FOxTROT: An International Randomised Controlled Trial Evaluating Neoadjuvant Chemotherapy for Colon Cancer in 1,052 Patients

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Neoadjuvant chemotherapy is established in many solid tumours but has had no previous large-scale evaluation in colon cancer. Patients with operable, non-obstructed colon cancer fit for chemotherapy and surgery with CT-predicted stage T3-4, N0-2 and M0, were randomised 1:2 to control (surgery then 24 weeks of FOLFOX) or novel (6 weeks of FOLFOX, then surgery, then 18 weeks of FOLFOX). RAS wild-type patients allocated to the novel arm had an optional sub-randomisation 1:1 to +/- panitumumab during the neoadjuvant phase. The primary endpoint was freedom from recurrent or persistent disease after two years, by intention to treat. Secondary endpoints included safety; histopathological stage, completeness of resection, DFS and OS. 1,052 pts were randomised between June 2008 and December 2016 at 85 centres in the UK, Denmark and Sweden. In the novel arm, 97% of patients received at least one cycle of neoadjuvant chemotherapy and surgery was attempted in 98% of cases. Neoadjuvant chemotherapy was well tolerated and gave marked histological downstaging, with lower pT (p<0.0001) and pN stage (p<0.0001) and fewer incomplete resections. (5% vs. 10%, p=0.09). Postoperative morbidity was reduced, with significantly fewer anastomotic leaks and complications requiring re-operation. Two year failure was less frequent after NAC (14% vs. 18%, HR=0.77, p=0.11), but this difference did not reach statistical significance. This is the first large-scale randomised controlled trial evaluating neoadjuvant chemotherapy for colon cancer. Neoadjuvant chemotherapy is safe and results in reduced postoperative morbidity, improved histological stage and a lower risk of incomplete resection. The observed improvement in two year failure rate fell short of statistical significance. Neoadjuvant chemotherapy for colon cancer improves surgical outcomes and can now be considered a treatment option; longer follow-up and further trials are required to assess its impact on long-term outcomes.

A Novel Artificial Intelligence Based Approach to the Diagnosis of Coeliac Disease, Based on T-Cell Receptor Repertoires

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Current testing strategies in coeliac disease (CD) (serology and histopathological examination of small intestinal endoscopic biopsies) require patients to eat adequate gluten prior to testing, meaning that significant numbers of likely undiagnosed gluten sensitive patients choose not to seek testing. Tests often give equivocal results, meaning that even after an endoscopy patients may remain unsure about whether they are gluten-sensitive. We aimed to develop a more robust and objective test that could identify CD in patients regardless of whether they consumed gluten. DNA was extracted from 60 formalin fixed paraffin embedded (FFPE) biopsy proven cases of CD (histologically Marsh 3B or 3C) and 45 control cases (no histological features of coeliac disease, no history of anaemia or abdominal bloating, biopsy taken for suspected gastro-oesophageal reflux disease). Bulk amplification of the T-cell receptor gamma and delta repertoires was undertaken with Lymphotrack™ and Biomed-2 kits (Invivoscribe) followed by next generation sequencing (Illumina). Novel methods for bioinformatic analysis were constructed in Python and R, using the IMGT database as a reference. We developed a novel algorithm to analyse T-cell receptor repertoires (TCRR), followed by dimensionality reduction and unsupervised nearest neighbour classification (e.g., clustering), grouping together cases with similar TCRR. By modifying the parameters, we could train/ supervise the algorithm to ensure that new cases were correctly clustered. Importantly, biopsies with normal histology from CD patients on a gluten-free diet without raised anti-TTG antibodies were classified as having CD. Our methodology has the potential to revolutionise the diagnosis of CD, so that it no longer relies on either the rather subjective opinion of a histopathologist or on sufficient gluten consumption by the patient. It is also applicable to FFPE biopsies and may, in future, be modified for use as a blood test.

Investigating the Potential of the Faecal Microbiome to Improve Colorectal Cancer Screening

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Colorectal cancer (CRC) patients have a different faecal microbiome to healthy controls. Microbiome data has been shown to improve the sensitivity of CRC screening in small studies collecting whole stool, often transported and stored refrigerated/frozen. We report the first study to pragmatically translate these findings to a national CRC screening programme, by analysing the microbiome directly from the faeces of processed NHS Bowel Cancer Screening Programme cards, stored and transported at room temperature. DNA was extracted from 1287 cards: 400 for which blood was not detected and 887 for which blood was detected (of which 250 had a normal colonoscopy; 88 a non-neoplastic condition; 291 adenoma; 258 CRC). A technical sub-study was conducted to assess stability by performing extraction replicates 6-23 months post-initial extraction. V4 16SrRNA sequencing was performed. There were no significant differences in bacterial communities of the extraction replicates, with a random forest model being unable to distinguish them, indicating stability. There were statistically significant differences in bacterial communities (weighted and unweighted UniFrac distances) between blood-positive and blood-negative samples and between all of the blood-positive colonoscopy-status groups, apart from the adenoma and non-neoplastic condition groups. The bacteria which differed significantly were in agreement with the existing literature, including an enrichment of *Fusobacterium*, *Parvimonas* and *Porphyromonas* in CRC. Preliminary random forest modelling has shown that samples can be classified with an improvement in accuracy up to 1.5 times baseline. We have demonstrated a method of performing population-level microbiome research. We have verified that the CRC-associated bacteria identified in research studies are present within a bowel cancer screening population. Preliminary work suggests that a microbiome-based model could be used to stratify screened patients.

Unmasking the Tissue Microecology of Ductal Carcinoma In Situ with Deep Learning

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Despite increasing evidence supporting the clinical relevance of tumour infiltrating lymphocytes (TILs) in invasive breast cancer, TIL distribution pattern surrounding ductal carcinoma in situ (DCIS) and its association with prognosis is not well explored. To characterize the tissue microecology of DCIS, we designed and tested a new deep learning pipeline, UNMaSk (UNet-IM-Net-SCCNN), for the automated detection and simultaneous segmentation of DCIS ducts using three patient cohorts. This new method achieved the highest sensitivity and recall over cutting-edge deep learning networks, as well as the highest concordance with DCIS identification based on CK5 staining. Following automated DCIS detection, spatial tessellation centred at each DCIS duct created the boundary in which local ecology can be studied. Another deep learning network was used to identify single cells including TILs. In a small sample set, we found a striking difference between pure DCIS cases and DCIS adjacent to invasive cancer. While pure DCIS cases had significantly more TILs, these TILs tended to co-localize less with DCIS compared to those with adjacent, infiltrating tumour, suggesting a more inflamed tissue ecology local to DCIS in tissue adjacent to invasive breast cancer. Thus, technological developments in artificial intelligence and digital pathology can enable us to quantify the spatial relationship between TILs and individual DCIS ducts, providing a new way to study immune response in DCIS.

A Display Evaluation for Primary Diagnosis using Digital Pathology

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As pathology departments around the world contemplate digitising for primary diagnosis, making an informed choice regarding which displays to purchase in the absence of defined minimum standards, is very challenging. To help inform the procurement of displays within our institution, and to help other departments make similar decisions, we conducted an evaluation of displays with a range of technical specifications. We invited histopathologists within Leeds Teaching Hospitals NHS Trust to take part in a survey evaluation of 8 short-listed displays. The displays were chosen from a range of vendors, and included consumer, professional and medical grade displays. After configuration was optimised, histopathologists blinded to make and model of the displays, were asked to review one H&E slide of a benign nevus on each display and give a score on a Visual Analogue Scale to indicate their preference in terms of image quality and display size. Thirty-eight pathologist participants took part. The preferred display was the most expensive display, which had the highest technical specifications (11.8MP resolution, 2100 cd/m2 maximum luminance, 1200:1 contrast ratio). The least preferred display was the least expensive display, which also had the lowest technical specifications (2.3MP resolution, 300 cd/m2 maximum luminance and 1000:1 contrast ratio). This experiment demonstrates a preference for medical grade displays with the highest technical specifications. As cost becomes implicated in procurement, significantly less expensive medical grade displays with slightly lower technical specifications may be the most cost-effective option.

Unbiased and Objective Artificial Intelligence Identifies Clinically Significant Diagnostic and Prognostic Features

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Purpose of the study: Digital pathology and image analysis can now quantify object-based and spatially resolved data from known histopathological features. However, cancer is a complex disease with multiple cellular interactions occurring within the tumour microenvironment. Undiscovered but clinically significant phenotypic patterns may exist within a patient sample. Artificial intelligence(AI) has the ability to identify such patterns by applying an objective and unbiased approach to the analysis. We utilise AI to predict diagnosis and prognosis in patients by quantifying features captured *a priori*. Furthermore we will describe how the iCAIRD project aims to translate such a deep learning approach into clinical reporting.
Methods: AI was applied to digitised images of patient samples to identify significant features in three clinical examples. 1) Stage II colorectal cancer (CRC) prognosis using immunofluorescence (IF) labelled whole slide images (WSI) (n=173). 2) Stage I & II CRC prognosis using H&E stained WSI (n=75). 3) Diagnosing bladder cancer recurrence from urine cytology samples labelled with IF (n=624).
Results: Automatically inferred phenotypes from H&E labelled stage I and II CRC patients, extracted with no human based annotations, predicted survival with an accuracy of greater than 95% and with an F score of 100%. 125 automatically reported features were extracted from IF labelled urine cytology samples and an AI workflow reported a sensitivity of 95% and a specificity of 70%. Unbiasedly extracted featured from IF labelled stage II CRC were analysed by AI and reported an AUROC of 0.94.
Conclusion: The use of automated image analysis and AI allow novel clinically significant features to be reported without the guidance of human-based training. This works paves the way for AI approaches to be translated into the clinic upon wider validation such as with the iCAIRD digital pathology initiative.

Contemporary Coronial Autopsy Practice in the NHS: An Audit

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An audit has been undertaken to review the coronial autopsy practice of a single histopathology consultant against the standards laid down by the Royal College of Pathologists 2002, National Confidential Enquiry into Patient Outcome and Death 2006, and Coroners Statistics Annual 2017 to identify areas of variance against national benchmarks. Outcomes from coronial autopsies facilitate trust morbidity and mortality review processes. The standard of post mortem practice and the accuracy of causes of death are therefore important factors to consider. The audit was approved by HM Coroner and registered with the Trust's audit department. Data were retrospectively collected from all post mortem examinations performed by a single consultant in 2017. The corresponding patient records were also consulted. The diagnostic value of histology and toxicology samples was explored. Discrepancies between suggested clinically and pathologically diagnosed causes of death were scrutinised. No attempt was made to reach objective judgements about the overall quality of the autopsy reports. 108 cases were audited of which 59% cases were community deaths and 41% of cases were hospital deaths. Compliance against most key standards was high, e.g. inclusion of supporting documentation, mandatory demographic details, external and internal examination. 52% of cases involved the retention of tissue samples; substantially higher than the national average for 2017 (23%). Histology confirmed the cause of death suspected during the post mortem examination (36%) or was essential in determining the cause of death (43%) in cases where tissue blocks were retained. Toxicology samples were retained in 24% of cases, compared to the national average (20%). It played a key role in determining the cause of death in 31% of cases. 60% of hospital deaths did not contain any clinical cause of death in medical notes. The clinician and pathologist given causes of death were similar in 33% of cases and significantly discrepant in 44% of cases. Histology remains a valuable component of autopsy practice. The autopsy sill has much to add when the cause of death cannot be determined clinically. Toxicology is very important in a small number of community deaths.

Enhancing Histopathology Laboratory Access Through Virtual Reality: Demonstrating Knowledge Gain and Potential Recruitment Benefits in Undergraduate Pathology Teaching

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Use of Virtual Reality (VR) technology is increasing, both in postgraduate medical training and in undergraduate medical education. Development of VR resources, previously restricted to games developers, is now accessible to educators as a result of decreasing hardware/software costs. Because of the challenges of exposing undergraduate medical students to histopathology working practices (and therefore enhancing their interest in the subject), we evaluated the use of VR, and 360 degree video, as an adjunct to pathology teaching on a medical undergraduate degree course at a UK-based University. Using 360 degree video, enhanced with a full narration audio, interactive hotspots and high-definition video pop-outs, we produced a fully immersive walk-through 'tour' of the histopathology laboratories within a large UK-based teaching hospital. The resource was produced with the intention of being deliverable using two methodologies: a traditional laptop/tablet device or using VR headsets for a fully immersive tour experience. As a trial, we delivered the 360 VR resource to separate cohorts of year 2 medical students. The value of the resource as a whole was assessed consecutively over 2 years, initially using entire year cohorts and in smaller 'focus groups'. An emphasis was made, by application of pre- and post-intervention questionnaires, on students' knowledge gain and their perceptions of 'immersiveness' and the relative values of non-VR versus VR-based delivery platforms. The relative value of high cost VR headsets vs low cost (cardboard) VR viewers was also assessed. Knowledge gain was slightly greater using ipads (0.65 vs 0.47), student's feelings of immersiveness were greater using VR (73% vs 27%), particularly when using high-end VR viewers.Notably 50% of students indicated that they would be more likely to consider pathology as a career post intervention. We thus demonstrate the delivery of an immersive histopathology experience to large cohorts at low cost.

Tips for Academic Pathology Trainees (APT): A Website offering Advice for Academic Pathology Trainees and Medical Students/Doctors Considering Histopathology as a Career

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Histopathology and academic pathology suffer from low recruitment and high attrition. Resources which promote histopathology have been produced by different organisations, but this makes them difficult to locate by medical students, junior doctors, or doctors from other specialties who are interested in histopathology. Resources offering advice and support to academic pathology trainees are usually delivered as face-to-face meetings; not all trainees are able to attend and the information may not be relevant to trainees until later in their careers.

We have created a website, "Tips for Academic Pathology Trainees (APT): a website offering tips for academic pathology trainees, doctors considering histopathology and medical students" (http://www.apt.virtualpathology.leeds.ac.uk). This serves as a single-site, permanent, universally-accessible, comprehensive set of resources for both medical students/doctors interested in histopathology and academic pathology trainees.

Content is divided into 12 main sections: Histopathology; Pre-PhD Fellowships; PhD Fellowships; Clinical Lectureships; Teaching; Networking; Literature-based skills; Finances; Professional Relationships; Patient and Public Engagement; General Research Skills; and Inspiration.

Users can submit suggestions, comments or questions via the email address: aptwebsite@pathsoc.org.

The website was officially launched in January 2019 and has been promoted via Twitter and email distribution lists. Two months post-launch, the website has had 809 users and 5131 page views, with most users viewing 6–7 pages per session. Users are from the UK, USA, Canada, India, Ireland and the Netherlands. Promotion is ongoing and website content will be regularly reviewed and updated. The high website-usage figures indicate that the website addresses a previously unmet need. We encourage you to use, promote and engage with the website.

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Taking Another Look: Using New Simulation Techniques to Evaluate Old Antibiotics

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Purpose of the study: Resistance to oral antibiotics recommended for common infections is increasing. Modern modelling/simulation techniques can assist in discovering new indications and dosing regimens for older antibiotics.

Objective: To design a framework to assess alternative treatments/novel dosing regimens for the treatment of common infections using simulation.

Methods: The framework development considered modern, robust model development techniques. Several simulation software packages were considered. Decisions were made using both microbiological and pharmacological expertise.

Summary of results: A framework was developed to systematically evaluate pharmacokinetic models of suitable quality from the literature for a selection of antibiotics. Once identified, models are evaluated for robust development methodology using quantitative and qualitative methods: 1) a numerical score: confidence in quality check based on key components (goodness-of-fit, NONMEM relative standard error, alternative software equivalent precision estimate, bootstrap analysis and simulation-based model diagnostics (SBMD)). 2) reviewer assessment of quality and relevance (raw data fit and potential for extrapolation to other populations). Highest quality models are then selected. This information can be combined with locally collected MIC data. Pharmacokinetic-pharmacodynamic simulations (R package mxlR), using the Monte Carlo method, can calculate the area under the curve of concentration/time graphs. This enables the generation of cumulative fraction response (CFR) values for sub-populations of the bacterial population at standard and non-standard doses, indicative of clinical success.

Conclusions: It is possible to conduct feasibility assessments for a number of commonly used oral antibiotics for varying indications and dosage regimens using limited resources. These assessments can indicate new potential in older antibiotics.

This research was supported by a Pathsoc Travel Grant.

Raising the profile of Histopathology among Medical Students: A District General Hospital Perspective (The Mid-Yorkshire Hospitals NHS Trust, An Associate Teaching Hospital Trust)

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Fewer medical students choose histopathology today as a career due to lack of awareness and poor perception. An ever-expanding curriculum, economic pressures and healthcare reforms negatively impact on pathology teaching. Profile raising engagement with medical students as part of existing clinical placements can increase appeal and demonstrates the value of the profession. Our experience shows how short integrated postings in a DGH Histopathology department can be delivered via vertical integration of pathology teaching during clinical placements (General Surgery, Special Senses, Oncology and General Medicine) in year 3 and above. This process of vertical integration allows the development of a spiral curriculum, which reinforces pathology concepts in year 3 and above. This approach also allows pathology to be revisited with an appropriate clinical context. Our short program includes a guided tour of the laboratory, specimen assessment in the cut up room, a posting-specific talk and a multi-header microscopy session. third year medical students have a day in the mortuary to observe post mortems and refresh their knowledge of anatomy and pathophysiology whilst encouraged to postulate a cause of death. Feedback is acted upon through peer review to improve teaching sessions and student feedback is shared to ensure continuous improvement. Students are invited to return in a voluntary capacity to shadow Consultants. We have introduced a Poster Presentation programme for students with the aim of presenting a poster at the annual Pathsoc scientific meeting. The programme has been assessed centrally and has been recommended to other DGH Histopathology Departments across Yorkshire. We are able to encourage students to view their learning in the context of both service delivery and clinical research.
We were awarded a Clinical Teaching Development Award 2014, nominated by students for Outstanding Clinical Team 2018 and awarded a Clinical Teaching Excellence Award 2019.

Adopting Digital Pathology into Clinical Practice: Perceived Advantages and Challenges

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Introduction: UK histopathologists are familiar with digital pathology in the setting of EQA schemes, and in an educational context. Few currently use this platform in the clinical diagnostic setting. As an NHS Department in the process of introducing a fully digital diagnostic histopathology service, we face challenges, both those that are described by others, and those associated with our own expectations and fears around digital pathology. These need to be recognised in order to increase the chances of success. At the start of our project we therefore sought to examine the perspectives, opinions and attitudes of the multiprofessional team most involved with introducing digital pathology.
Methods: A survey was sent out to members of the local Digital Pathology Steering Group. The survey incorporated questions to ascertain the opinions and attitudes of the team to the transition to digital pathology, and sought to identify areas of potential concern.
Results: 9 people completed the survey. Responses were positive with regard to the transition as a whole, with a general consensus about projected benefits for improved workflow, patient safety, and workforce (including network working). There was no real perceived concern about the process of transition in terms of time taken to become confident in digital reporting, or to report digitally compared with glass slides. Whilst generally the improved ease of sharing of cases was seen as benefit to patients and for education, concern was raised that facilitation of access to specialist histopathologists for second opinion might significantly increase referral work. It will be important that clear pathways for access to opinions are established and to ensure time is appropriately recognised and funded.
Conclusion: Overall the attitudes and opinions around the transition to digital pathology were positive. The perceived challenges are not insurmountable, and as identified at this stage they can be addressed.

Inspiring the Next Generation: The National Academic Trainees' Network

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Purpose of the study: Over the last 15 years there has been a well-documented decrease in academic pathology capacity in the UK. Initiatives such as the CM-Path programme by the National Institute of Cancer Research are designed to remedy this decline. We recognised a lack of training in non-technical skills for junior academic pathologists and the lack of an effective network for sharing success, advice and opportunities. This led us to create the National Academic Trainees' Network (NATN).
Methods: The network meets three times per year and comprises a networking dinner and inspirational speaker with a full day of tutorials and workshops the following day. Sessions have included: advice on grant writing, social media strategy, legislation including research ethics and working with human tissue, lay communication, intellectual property, three-minute thesis presentations, research impact and the introduction of a mentoring scheme. We evaluated feedback from the meetings and the content of the network WhatsApp group.
Results: Feedback was available from eight of the ten NATN meetings that have taken place to date. The mean attendance was 22 delegates per meeting. Academic clinical fellows, lecturers and trainees from the alternative academic pathway were equally represented. Across all meetings the mean weighted average rating of all sessions was 4.28/5. A common theme from the feedback was that trainees found the networking opportunities and peer support elements of the meetings very useful. A NATN WhatsApp group has been active for 5 months. This has 33 members and has been used for advertising conferences and resources, discussing grant application logistics and developing future NATN sessions.
Conclusions: To our knowledge the network is unique amongst academic medical specialities. Trainees have given favourable feedback and the network appears to offer training not routinely provided by academic training programmes.

Addendum Pathology Reports: Amended, Corrected or Supplementary? Does it matter?

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Background: Addendum pathology reports are issued subsequently to a final report when additional details or a change to the report is required. Their rates may be used as a departmental key performance indicator of accurate reporting. However, not all such reports are due to departmental inaccuracies. Published guidance from the Faculty of Pathology, Royal College of Physicians of Ireland recommends that addendum reports be subcategorised into amended, corrected and supplementary types. Of these, amended reports are those which contain a change to the report significant enough to affect patient management such as an amendment to the diagnosis or stage. Corrected reports are used to correct typographical or other minor errors. We performed this study to analyse our amended reports to see if we could re-classify them using the above criteria focusing on the possible benefit of separating amended from corrected reports.
Methods: All amended reports issued by our department over a three month period were identified, reviewed and reclassified using the above criteria.
Results: A total of 19,523 specimens were reported over the study period with 63 amended reports issued (0.3%). When reclassified, only 18 (28%) of these amended reports detailed a change in diagnosis/stage from the original report. Other reports corrected minor errors and were better classified as corrected (57%), a few contained new information and should have been classed as supplementary (9%).
Conclusions: Clearer categorisation may help receiving clinicians recognise the importance and clinical relevance of amended reports as distinct from corrected reports. In addition it would allow departmental reporting error rates to be more accurately represented as only a quarter of issued amended reports actually contained true reporting errors in our cohort.

Glutaminase Expression Predicts Recurrence in Ductal Carcinoma *In Situ*

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Purpose of the study: Ductal Carcinoma in Situ (DCIS) shares many morphological and molecular similarities to invasive breast cancer (IBC) and precise predictors of DCIS recurrence and progression are still lacking. In order to meet increased metabolic demands of growth and proliferation, many cancers reprogram their metabolism to rely on glutamine. Glutaminase 1 (GLS1) is a key enzyme, which converts glutamine to glutamate in the glutaminolysis process. GLS1 expression is upregulated in IBC, but has not yet been studied in DCIS. In this study, we evaluated the expression of GLS1 in a large cohort of DCIS and assessed its prognostic significance.
Methods: In this retrospective study, GLS1 expression was assessed immunohistochemically in a large, well characterised DCIS cohort, consisting of 779 pure DCIS cases and 239 cases of DCIS-mixed with IBC. GLS1 expression was correlated with clinicopathological parameters, and outcome analysis was evaluated using local recurrence free interval (LRFI).
Summary of results: GLS1 expression was associated with features of high-risk DCIS, including symptomatic presentation (p=0.04) and tumours that were higher grade (p=0.014), ER negative (p<0.001), PR negative (p=0.003), as well as high Ki67 (p=0.036) and high hypoxia inducible factor 1α (HIF1α) (p<0.001) expression. GLS1 expression was higher in DCIS mixed with IBC compared to pure DCIS (p=<0.001). GLS1 expression was an independent prognostic feature in predicting both invasive (HR=4.1, 95%CI 1.4 to 12.0, P=0.012) and DCIS recurrence (HR=19.8, 95%CI 4.3 to 92.0, P<0.001).
Conclusion: We show for the first time, that GLS1 may play an important role in DCIS progression as well as predicting recurrence in DCIS patients. This study also highlights the potential therapeutic role of GLS1 inhibitors in DCIS patients.

Over Expression of Retinoid X Receptor Gamma (RXRG) Predict Good Prognosis in Oestrogen Receptor Positive Breast Cancer

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Background: Breast Cancer (BC) is globally one of the most prevalent malignancies and a leading cause of cancer-related death. Retinoid X Receptor Gamma (RXRG) is a member of the nuclear receptor superfamily, which interacts with other nuclear receptors and plays a role in tumour suppression. This study aims to investigate the prognostic role of RXRG in BC.
Methods: RXRG protein expression was evaluated using a large well-characterised BC cohort (n=923) prepared as tissue microarrays. The association with different clinicopathological parameters and patient outcome were investigated. Prognostic significance of RXRG mRNA expression was also assessed using breast cancer gene miner (bc-GenExMiner v4.2).
Results: High nuclear RXRG expression is associated with good prognostic features including good Nottingham Prognostic Index group (p<0.05), lower histological grade (p=0.04) and smaller tumour size (p=0.036). Strong positive associations were observed with oestrogen receptor (ER) positivity and ER-related biomarkers: GATA3, FOXA1, STAT3 and MED7 (p<0.00001), and reduced expression of the proliferation marker Ki67 (p=0.014). RXRG overexpression was associated with longer BC-specific survival (p<0.0001) and less probability for the development of distant metastasis (p=0.003). In ER-positive tumours, high expression of RXRG showed significant survival advantage regardless of adjuvant systemic therapy (p=0.04). RXRG expression is an independent prognostic factor associated with improved survival, particularly in ER-positive BC. In the external validation cohorts, RXRG mRNA expression was associated with improved patients' outcome (p=0.025). Differential gene expression evaluation identified ER signalling pathway as the principal predicted master regulator of RXRG expression (p=0.005).
Conclusion: The findings support the proposed role for RXRG as a prognostic marker in ER-positive BC. Exploring the utility of RXRG as a potential therapeutic marker is warranted.

Keratin 24 (KRT 24) Confers Poor Patient Outcome in Invasive Breast Cancer

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Background: Keratin (KRT) 24 is cytoskeletal protein playing a major role in the formation of the intermediate filaments that provide mechanical stability. The components of the cytoskeleton mediate tumour cell migration, invasion and metastasis. Metastasis is the major cause of breast cancer (BC) related deaths. Through stringent bioinformatics analysis we identified KRT24 overexpression as strongly associated with poor patient outcome; however, its role in BC remains unclear. The study investigates the clinicopathological significance of KRT24 at transcriptomic and proteomic levels using large cohorts of BC patients with long term follow-up.
Methods: KRT24 mRNA expression was assessed in the METABRIC (n=1980) cohort and externally validated in BC Gene miner v4.0. Primary BC tissue microarrays (n=827) were immuno-stained for KRT24 and correlated with clinicopathological features, patient outcome and with other BC related markers.
Results: KRT24 mRNA was associated with poor prognostic features, including high histological grade, ER negativity, HER2 positivity and is overexpressed in luminal B and HER2+ subtypes (all; p<0.05). KRT24 protein expression was significantly associated with features of aggressive phenotype including HER2-positivity (p=0.003), cycline E (p=0.026), N-cadherin (p=0.012), and epidermal growth factor receptor (EGFR; p=0.043) and basal phenotype (p=0.016). Using BC Gene miner (n=3163), high KRT24 mRNA expression was associated with poor patients' outcomes (p=0.027). High KRT24 protein expression was associated with poor BC-specific survival (p=0.029). Cox proportional multivariate analysis revealed that high KRT24 is a predictor of shorter BC-specific survival, an independent of other clinicopathological factors (p=0.023).
Conclusion: This study confirmed the association between KRT24 expression and poor prognostic features and metastasis related biomarkers; results warranting further functional validation.

CD133 Over-Expression in Breast Cancer: A Marker of Poor Prognosis

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Purpose: The cancer stem cell marker, CD133, is associated with poor prognosis in various solid tumours, but its role in invasive breast cancer (BC) remains unclear. Thus, this study aims to evaluate the prognostic importance of CD133 expression utilising large well characterised BC cohorts with long-term follow up.
Methods: CD133 mRNA expression were assessed in the METABRIC cohort and externally validated in BC Gene miner v4.0. Primary BC tissue microarrays (n=687) were immuno-stained for CD133 and correlated with clinico-pathological features, patient outcome and with other stem cell markers.
Results: CD133 protein expression showed a positive correlation with CD133 mRNA (Spearman's coefficient 0.505; p<0.00001). CD133 immunopositivity was observed in the cytoplasm / membrane of invasive cancer cells. Similar to mRNA expression, high CD133 protein levels were associated with high grade, larger tumour size, poor Nottingham Prognostic Index, HER2 positivity and hormonal receptor negativity (all; p<0.001). CD133 protein overexpression was significantly correlated with features of aggressive phenotype including basal cytokeratins CK5/6, CK14 and CK17, Epidermal Growth Factor Receptor (EGFR) and proliferation marker Ki67 (all; p<0.05). A strong positive association with other BC stem cell markers such as CD24, CD44, SOX10, and ALDHA3 were observed (p=0.020). High expression of CD133 protein was associated with shorter BC-specific survival in the whole cohort (p<0.001) and Her2+ subgroup (p=0.04). Cox proportional multivariate analysis showed that that CD133 protein expression was an independent indicator of shorter BC-specific survival (p=0.038).
Conclusion: This study provides evidence for the prognostic value of CD133 in invasive BC particularly in the aggressive HER2+ subtype of BC, and is, therefore, a potential therapeutic target.

Flap Endonuclease 1 (FEN1) is a Prognostic Biomarker in Ductal Carcinoma *In Situ* (DCIS)

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Background: Carcinogenesis could be driven by impaired DNA repair which clinically promotes aggressive behaviour in breast carcinoma. Damaged DNA bases can be removed accurately by base the excision repair (BER) pathway. FEN1 is a major component in the BER which has important roles in genomic stability maintenance through the rescue of stalled replication forks, maintenance of telomere stability and apoptosis. FEN1 also plays a role in replication by controlling the Okazaki fragment maturation. High expression of FEN1 is associated with poor outcome in invasive breast cancer (IBC), however, it is not confirmed in the pre-invasive stage. We hypothesised that FEN1 overexpression is an early event in breast cancer pathogenesis. The aims are to assess the role of FEN1 in DCIS with the potential to predict DCIS progression to invasive disease.
Methods: 779 pure DCIS and 239 mixed DCIS/IBC cases were constructed. The expression of FEN1 was assessed using immunohistochemistry and correlated with the clinicopathological parameters and patient outcome.
Results: In a pure DCIS cohort, high expression of FEN1 was associated with higher DCIS grade (P<0.0001), size >20mm, P=0.008), presence of comedo necrosis (P<0.0001), ER and PR negative tumours (P<0.0001) and high expression KI67 labelling index (>14%) (P<0.0001). DCIS component of the mixed cases showed higher expression compared with DCIS in the pure DCIS cohort (P=0.019) but showed significantly lower expression compared with the coexisting invasive component (P<0.0001).
Conclusion: These results suggest that overexpression of FEN1 is associated with aggressive DCIS. We concluded that overexpression of FEN1 in pre-invasive DCIS is linked to the invasive breast cancer, but it's not linked to local recurrence. FEN1 in DCIS is a poor prognostic factor that can potentially help in in situ risk stratification.

High SLC1A5 Expression Predicts Resistance to Endocrine Therapy in ER+ Breast Cancer via Interacting with Metabolic Pathway

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Background: Identification of effective and reliable biomarkers to predict the efficacy of endocrine therapy and understanding the molecular pathways that contribute to the development of endocrine resistance are of crucial importance to the management of oestrogen receptor positive (ER+) breast cancer (BC). Glutamine-dependence is an established hallmark of cancer and the transport of glutamine into the cell via SLC1A5 has an emerging importance as a diagnostic and therapeutic target.
Methods: We investigated the biological impact of SLC1A5 expression in a clinical samples of ER+ BC at the mRNA, using METABRIC and KM-Plotter datasets, and the protein levels using immunohistochemistry in a large annotated series of ER+ BC with long-term follow-up. Additionally, bioinformatics analysis were used to study the interacting networks of SLC1A5, and its biological processes and enriched pathways.
Results: SLC1A5 expression was associated with poor clinicopathological parameters (vascular invasion, large tumour size and high grade; (P<0.05), and poor clinical outcome (P<0.05). SLC1A5 expression also significantly correlated with tamoxifen resistance in patients treated with adjuvant tamoxifen monotherapy (P<0.05). In silico analysis, showed the majority of SLC1A5 correlated-genes within the biological interacting network were were significantly enriched within the metabolic and MAPK signalling pathways. In the top-25 genes of interacting network with SLC1A5, TALDO1 was involved in metabolic pathway and associated with poor clinical outcome in ER+ BC and resistance to endocrine therapy.
Conclusion: This study shows that the glutamine transporter SLC1A5 could act as potential predictive biomarker of poor benefit from endocrine therapy in BC and a potential therapeutic target

The Prognostic Significance of PDZ Domain-containing 1 (PDZK1) in Invasive Breast Cancer and its Association with ER Heterogeneity

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Background: Endocrine therapy is the standard systemic treatment for oestrogen receptor (ER) positive breast cancer (BC), however approximately 40% of these patients develop recurrence. ER intra-tumour heterogeneity is a potential reason for resistance to endocrine therapy and subsequent recurrence. The protein PDZ domain-containing 1 (PDZK1) is encoded by an oestrogen-responsive gene present in BC cells. The study aims to investigate the prognostic role of PDZK1 and its association with ER heterogeneity.
Methods: PDZK1 mRNA expression was evaluated [BC Gene miner and the METABRIC cohort] against clinicopathological variables and patient outcome. Full-face BC sections were stained for ER and PDZK1 using immunohistochemistry (IHC). Intra-tumoural heterogeneity was assessed on high definition digital images divided into 4 quadrants and an association between ER and PDZK1 expression was also assessed.
Results: In the METABRIC cohort, PDZK1 mRNA expression was associated with low histological grade, smaller tumour size, good Nottingham Prognostic Index, hormone receptor positivity and HER2 negativity (all p<0.001). High expression of PDZK1 mRNA was also associated with longer BC-specific survival (p<0.001). Using BC Gene miner, PDZK1 mRNA expression was associated with improved patients' outcome (p=0.001). However, PDZK1 at protein level displayed scatter and cluster heterogeneity. Association between ER and PDZK1 expressions revealed that 18.75% of cases showed similar expression percentage and intensity, 43.75% showed an association with percentage, while 6.25% showed an association with intensity. Distribution pattern, cluster and scatter heterogeneity of ER and PDZK1 were also shown to be associated.
Conclusion: This study provides evidence for the prognostic value of PDZK1 mRNA in invasive BC. The heterogeneous pattern of expression of both ER and PDZK1 further establishes the link between PDZK1 and ER; warranting further validation of its clinical utility.

Transcriptomic Profiling of Triple Negative Breast Cancer Identifies SPDYC as a Novel Independent Predictor of Outcome

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Background: The lack of robust prognostic markers for the aggressive triple negative breast cancer (TNBC) leads to unselective treatment of patients. Transcriptome profiling of TNBC identified several subclasses but no single gene was reported as a risk classifier. In this study, using next generation sequencing (NGS) we identified SPDYC as a strong prognostic marker in TNBC. SPDYC is a member of the speedy\Ringo cyclin- dependent kinase (CDK) family, which promotes progression through cell cycle by binding and activation of CDK1 and CDK2. We further examined the clinicopathological significance of SPDYC at the protein level using a large annotated TNBC cohort.
Methods: Supervised artificial neuronal network (ANN) analysis of gene expression was applied on RNA-Seq depository utilising the HiSeq2500 instrument (Illumina, Inc) to identify differentially expressed transcripts with respect to distant metastasis-free interval (DMFS) and breast cancer specific survival (BCSS). Primary TNBC tissue microarrays (n=305) were immuno-stained for SPDYC and correlated with clinicopathological features and patient outcome.
Results: High SPDYC mRNA expression was significantly associated with shorter BCSS (P=0.014) and DMFS (P= 0.018). SPDYC mRNA expression was also independent poor prognostic transcript for BCSS and DMFS (both; P<0.01). High SPDYC protein expression in tumours was observed in 234 out of 305 cases (59%, H score >90). High SPDYC protein expression was significantly associated with poor BCSS and DMFS (both; P<0.01). Cox proportional multivariate analysis revealed that high SPDYC expression is a predictor of shorter BCSS and DMFS, independent of other clinicopathological factors (P=0.015).
Conclusion: Our study identified SPDYC as an independent predictor for prognosis and outcome in TNBC thus can be a potential guide for therapeutic decision

Methods of Nucleolar Assessment in Invasive Breast Cancer and their Prognostic Significance

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Background: Size and number of nucleoli are attracting considerable attention for its potential role in cancer development and progression. Analysis of prominent nucleoli is considered as one of the several important considerations for cancer diagnosis and progression. This study aims to investigate (a) methods of nucleoli scoring of optimal performance (b) their prognostic significance in invasive breast cancer (BC) and (c) the added value of nucleoli scoring to enhance performance in BC grading.
Methods: Hematoxylin and eosin (H&E) stained sections from invasive breast carcinoma cohorts with long-term clinical follow-up split into training (n = 400 cases) and validation (n =1200 cases) set. Four different scoring methods were applied to the training set to identify the most optimal objective and reproducible method associated with a high prognostic value.
Results: Among the four methods used, counting nucleoli in one high power field (hot spot) provided a higher significant association with outcome and highest concordance rate [intraclass correlation coefficient = 0.988]. Prominent nucleoli score in 10 hpf , 5 hpf as well as evaluation of nucleoli in 20% of the tumour were practically less reproducible, more subjective and showed lower concordance rates than nucleoli score in a hot spot. Within the validation set, high nucleoli score were associated with younger age, larger size, higher tumour grade, advanced stage, estrogen receptor negativity, progesterone receptor-negativity and HER2 positivity as well as shorter survival, shorter time to distant metastasis and shorter recurrence-free interval (p =0.000004, p= 0.00001 and p=0.008, respectively). Also adding nucleoli score as a part of BC grading components, grade showed highly significant association with survival (p = 7.48 × 10-13).
Conclusion: Nucleoli scoring in hot spot as an assessment method for nucleoli in H&E stained full-face sections is a reproducible and practical method to predict tumour behaviour

The Prognostic Significant of the Stem Cell Marker ALDH1A1 in Breast Cancer

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Background and aims: Aldehyde dehydrogenase family 1 member A1 (ALDH1A1) has been identified as a cancer stem cell marker in several cancers. In this study, we evaluated the prognostic and biological significance of ALDH1A1 in breast cancer (BC).
Methods: ALDH1A1 was assessed using immunohistochemistry in a large (n=900) well-characterised annotated series of early-stage BC patients with long term follow-up prepared as tissue microarrays. Expression was also characterized using full-face sections of excision specimens (n=28).The associations between ALDH1A1 and clinicopathological parameters and patients outcome as well as with other relevant BC stem cell markers (CD44,CD24, CD133, SOX9,SOX10,EPCAM, and CD133) were determined in the different molecular subtypes.
Result: ALDH1A1 showed homogenous expression in cytoplasmic tumour cells. High cytoplasmic ALDH1A1 expression was associated with poor prognostic features including high grade, high mitotic count, increased nuclear pleomorphism, poor Nottingham Prognostic Index, high nodal stage, and highly proliferative ER+ and Triple negative (TNBC) subtypes and ki67 (P<0.05). High ALDH1A1 expression was significantly associated with poor BC specific survival (BCSS) for 20 year (P=0.000). Based on molecular classes, high ALDH1A1 was significantly associated with poor BCSS (20 year) in highly proliferative ER+ and TNBC subtypes (P=0.04 and 0.002 respectively). High expression ALDH1A1 also predicated the response of chemotherapy in TNBC (P<0.05). ALDH1A1 was positively correlated with the expression of the stem cell markers EPCAM and SOX9 (P<0.05).
Conclusion: ALDH1A1 expression is associated with poor prognostic characteristics in BC particularly in luminal B and TNBC. Moreover, ALDH1A1 can predict the response of the chemotherapy in TNBC.

Up-Converting Nanoparticles as a Tool for Histopathological Tissue Evaluation with Multiplexing and Machine Learning Potential

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In the field of histopathology, pathologists diagnose patients by assessing imaged tissues. Even with the pathologists' trained eye, there is a great risk for misdiagnosis. For decades haematoxylin and eosin (H&E) stain has been a standard way to visualise morphology of the cells. It is also common to detect proteins using a DAB chromogenic stain and combine it with a single counterstain to visualise cell nuclei. However, this method suffers from narrow dynamic range, problems with quantitation and difficulties with multiplexing and co-localisation. Fluorescent IHC techniques generate a more quantitative readout but suffer from photobleaching. Here we present that the use of up-converting nanoparticles (UCNPs) allows to overcome problems associated with commonly used imaging techniques. Novel luminescent UCNPs were used together with a prototype instrument to image selected markers, e.g. Her2, in the human tissue. Formalin-fixed paraffin-embedded human colon and breast cancer tissues were sectioned and stained using autostainer. UCNP fluorescence imaging of the human tissue sections was compared with a standard DAB based IHC. Pulsed excitation and gated detection were explored to improve the scanning speed. UCNP and H&E co-staining and co-imaging were also investigated. Images obtained with our novel device clearly show that developed by us antibody-UCNP conjugates can be used to successfully stain human tissues. Brightfield images show that UCNPs are not visible in white light and hence do not interfere with standard tissue evaluation by a pathologist. Additionally, brightfield and luminescent images can be merged to provide better understanding of tissue morphology. Emerging field of UCNPs opens up new possibilities. Staining solutions and a novel device developed by us give hope for more accurate diagnosis by keeping the advantage of H&E staining and combining it, in one image, with luminescent data, ideal for generating ground truth for machine learning algorithms.

Evaluating Steroid Hormone Receptor Profiles in Triple Negative Breast Cancer and their Association with Clinical Outcome

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Triple negative breast cancer (TNBC) accounts for 15% of all breast cancers diagnosed. TNBC lacks expression of the three receptors which are required for response to the current targeted endocrine and biological therapeutics - oestrogen receptor alpha (ERα), progesterone (PR) and human epidermal growth factor receptor 2 (Her2). A targeted treatment option for TNBC remains a significant unmet clinical need in breast cancer care. ERα and PR belong to the steroid subfamily of nuclear receptors, which includes the glucocorticoid receptor (GR), mineralocorticoid receptor (MR), androgen receptor (AR) and oestrogen receptor beta (ERβ). These receptors share functional similarity to ERβ and PR, and so if expressed, targeting these steroid receptors may offer an alternative therapeutic target for TNBC. This study explores the hypothesis that expression of other steroid receptors may predict prognosis in TNBC. The profiles of GR, MR, AR and ERβ were determined in 39 TNBC cases. TNBC tumours are heterogeneous, and steroid receptors have variable expression in different cell types. To control for this, QuPath software[1] was used to specifically identify tumour regions in tissue sections immunostained for each steroid receptor. The proportion of tumour cells expressing each receptor were quantified. Correlations between receptor status and patient survival were determined using clinical data for each sample. Kaplan Meier survival analysis revealed that high GR expression and low AR expression were linked to poor outcome in our TNBC cohort. Pairwise analysis identified no correlation between receptor expression in TNBC tumour cells. Future studies will determine the effect of pharmacological modulation of AR and GR alone and in combination to determine the effect on TNBC cell fate. Through this, we hope to identify alternative therapeutic strategies to treat TNBC.
[1]Bankhead P. et al. (2017) QuPath: Open source software for digital pathology image analysis. Scientific Reports.

Audit of Breast Fine Needle Aspiration Cytology Diagnoses at a Large Teaching Hospital Unit

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Purpose of the study: Fine needle aspiration (FNA) of the breast is used as part of triple assessment of patients presenting via the NHS Breast Cancer Screening Programme (NHSBSP) and the symptomatic breast service. Cases are coded as C1-C5 (inadequate-malignant). An audit was completed to evaluate the accuracy of FNAs at Hospital A.
Methods: Information was collected for all FNAs from July-December 2016 using pathology reports.
Summary of results: Coding was; 49%, 38%, 2%, 1%, 4%, 6% for C1-C5 and other respectively. Twenty-two percent had further testing. Of these, the cytological diagnosis was correct in 86%. The sensitivity and specificity was 65% and 98% respectively. The false-positive rate for C4 was 25% and the PPV for C5 was 100%. Considering only clinically/radiologically suspicious lesions, the rate of inadequate samples dropped to 3%. The rate of C1 diagnoses is larger than documented (7.8-32%) and NHSBSP recommendations. However, this drastically decreases for suspicious lesions. Sensitivity is lower than other reports (71.5-97.5%) and NHSBSP recommendations. Cytological diagnoses and diagnosis of suspicious/malignant lesions show good to perfect accuracy.
Conclusions: Hospital A produces a high number of inadequate/C1 FNAs and has a low sensitivity, but performs well diagnosing malignant lesions. It could be considered that reserving FNA for more clinically appropriate patients could improve standards.

Is Bigger Always Better? X-ray Guided Biopsies in Breast Cancer Screening

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Introduction: In March 2018 the regional breast cancer screening service changed first line X-ray guided biopsies from 12 gauge vacuum assisted biopsy (12G VAB) to 9G VAB. Whilst on the face of it, more tissue seems better as it is more likely to be a representative diagnostic sample, there were two concerns raised (i) With bigger diameter biopsies the standard practice of 4 levels for calcifications may not be sufficient. Further levels may be required on an increased number of cases with implications on turn around times, laboratory and pathologist workload and logistical issues with clinic appointments for final results to be ready. (ii) More sample tissue could result in increased pick up of incidental B3 lesions (e.g. in situ lobular neoplasia (ISLN)) requiring further sampling, increased patient anxiety and radiology and pathology workload.
Methods: We measured the indication for sampling, size of tissue sampled, number and % requiring extra work, type of extra work and the diagnosis and B-codes and compared with a 12G VAB cohort.
Results: The change in diameter of 12G to 9G is 1 mm to 1.5 mm resulting in an increased volume of 193%. In March-November 2018 451 9G VABs were taken. 273 (61%) were taken for calcification and 178 (39%) for mass/asymmetry/nodule/density. For those with calcifications 65 (24%) needed further work, 49 (18%) required additional levels as calcifications not adequately seen initially. 10 biopsies for calcification (4%) ended up as B3 due to the prevalence of incidental ISLN. In comparison, of 273 consecutive 12G VABs for calcification from 2017 40 (15%) required additional levels and 5 (2%) had incidental ISLN.
Conclusions: This study has highlighted that additional levels are needed in a high percentage of calcification cases and that incidental ISLN is not a rare occurrence. However, this study has also shown that the increase in cases requiring additional levels and in incidental ISLN is not as big as concerns initially suspected.

Breast Implant Associated Anaplastic Large Cell Lymphoma (BIA-ALCL): A Local Case Series and the Introduction of a Proposed TNM Staging Criteria in Reporting

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Purpose of study: BIA-ALCL is a relatively new entity, first recognised in 1997, and a TNM staging system has recently been proposed (J Clin Oncol. 2016;34(2):160-8). There is currently no mention of BIA-ALCL in the RCPath datasets for the reporting of breast cancer or lymphoma. We aimed to review cases reported in a large teaching hospital in relation to the proposed TNM staging system, along with evaluation of follow-up data for these patients.
Method: Review of cases of BIA-ALCL reported at Leeds Teaching Hospitals NHS Trust (LTHT) and retrieval of follow-up data from the hospital EPR system.
Results: We identified three patients diagnosed with BIA-ALCL, comprising five pathology specimens, including seroma fluid, capsulectomy specimens, lymph nodes and recurrence samples. These have all been reviewed and diagnosis agreed by both the Histopathology Department and Haematological Malignancy Diagnostic Service (HMDS) provided at LTHT. The patients presented at different pathological stages of the recently proposed TNM staging criteria – including T1, T2 and T4 staging. The T2 case also had nodal disease, and the T4 case has since had further surgery for recurrent disease. Both of these two ladies received chemotherapy post the initial surgery.
Conclusions: Our limited case series supports the proposed TNM staging, and patients with a higher T stage are more likely to have metastatic disease or recurrences requiring on-going follow-up and management. At LTHT we have introduced the TNM staging in our histology reports as this is purported to provide improved prognostic information to our surgical and haemato-oncological colleagues on overall survival, recurrence rates, and the requirement for adjuvant chemotherapy and radiotherapy. We will continue to use the proposed TNM staging system in our reports locally, and would also encourage its use in other centres to allow data collection and comparison going forward. We would also encourage the College to introduce guidelines for assessment and reporting, along with the TNM system in future breast and lymphoma datasets.

The Handling and Reporting of Breast Cavity Shave Specimens: A National Survey of UK Practice

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Purpose of the study: Cavity shave (CS) specimens are integral to breast conservation surgery and contribute significantly to breast pathology workloads. We investigated current UK sampling and reporting practices for CS taken during wide local excision (WLE) surgery with reference to existing national guidelines.
Methods: UK breast pathologists were sent an online questionnaire during May – July 2018 followed by 3 reminders.
Summary of results: 110 pathologists completed questionnaires (response rate 20%). 7% of respondents receive CS with all WLE specimens. Targeted CS specimens are more common than 4 standard CS (inferior, superior, lateral, medial). The average CS weight is estimated as less than 10g (59%) and 10-20g (35%). The commonest sampling methods are to embed all tissue (39% always; 47% often; 13% sometimes) or sample areas of macroscopic abnormality only (15% always; 7% often; 34% sometimes). Few shave the new margin only (4% always; 0% often; 6% sometimes). Most pathologists ink the new margin only (54%) and slice perpendicular to this (89%). Commonest definitions for complete local excision for invasive cancer were 1mm (73%); not at ink (14%); 2mm (5%) and for high grade DCIS were 1mm (52%); 2mm (33%); not at ink (6%). 46% of pathologists felt more specific national guidelines for CS would be helpful.
Conclusions: The current sampling, handling and interpretation of cavity shave specimens appears to be inconsistent. Updated national guidance could assist UK breast pathologists and standardise clinical practice.

Adenomyoepithelial Adenosis of the Breast Presenting as a Palpable Mass Mimicking Malignancy on Imaging

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Introduction: Adenomyoepithelial adenosis (epithelial myoepithelial adenosis) is an extremely rare lesion, with asymmetry, irregular margins and calcifications mimicking malignancy on imaging. These biphasic lesions includes spectrum of adenomyoepithelial adenosis, adenomyopithelioma and their malignant counterpart. Here we report a case of adenomyoepithelial adenosis proved to be difficult to diagnose pre-operatively.
Case History: A 46yr old woman presented with right breast distortion and mass like lesion was diagnosed as an intraductal papilloma with florid epithelial hyperplasia in 14G core biopsy and followed by local excision.
Pathology: The lesion was 24mm in diameter had ill-defined margins, nodular architecture with areas reminiscent of intraductal papilloma. In places there were small sized tubules focally giving microcystic appearance, composed of two cell type; an inner layer of ductal cells and outer layer of CK14 and CK5/6 positive myoepithelial cells. Areas with apocrine change and columnar cell change were also present.
Discussion: Our case confirms the rarity of the lesion and illustrates the difficulty to achieve a pre-operative diagnosis. As cited in literature, this is a benign lesion which may progress to malignancy, with few cases that were associated with intraductal carcinoma and malignant myoepithelioma. No correlation was found between tumour size and progression of malignancy. The reported cases were <50mm and predominantly a disease of young women as in our patient. It is important to ensure excision is complete because of the tendency to recur with inadequate excision margins.
Conclusion: This is a rare lesion with limited data showing an association with malignant progression and local recurrence, which needs complete excision with adequate margins. Accurate diagnosis requires immunohistochemistry for basal/myoepithelial markers.

Case Series to Highlight the Clip Site Reaction in Breast Pathology

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Introduction: Biopsy marker placement is an important step in the breast biopsy procedure mostly in screen detected lesions. These marker clips are important to indicate the location of lesions with increased rate of negative margins. The markers are most commonly metallic in various shapes but more recently new types of clips have become available on the market. They may be accompanied with embedding material such as collagen, hydrogel or PVA polymer. This material helps to decrease clip displacement and can aid ultrasound identification of the clip site. Little is known of the changes induced by the modern type markers in breast tissue. Therefore, we decided to review our series of cases demonstrating the clip site reaction in breast tissue and its relation with the targeted lesion removed with both vacuum and surgical type of specimens. In addition, we have looked at the interval of time between clip insertion and tissue reaction.
Case series: A series of breast excisions following a biopsy containing a clip site reaction were collected. These were characterised by a clear cut cavity, which was associated with a multinucleated giant cell reaction and surrounding fibrosis. Some contained jelly-like material.
Discussion: The tissue reaction at the clip site is dependent on the clip material and the interval of time from clip insertion to resection. In our series the typical changes were noted 3–6 weeks after clip insertion. Awareness of tissue changes due to the clip is helpful for several reasons: 1. Accurate assessment of breast specimen resected for small screen detected lesions; 2. Confirming presence of clip site in the pathology report is valued by surgeons as proof of precise localisation; 3. Increases patients satisfaction as it provides reassurance the targeted breast lesion has been removed. **Conclusion:** This case series has highlighted the histological appearances of clip sites. This is important in aiding to confirm the correct site of sampling.

A Dedicated Breast Cancer Prevention Clinic: Reducing Breast Cancer Incidence and Identifying Biomarkers Indicative of Therapeutic Response

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Purpose of study: NICE recommends breast cancer preventive therapy for women at high or moderate risk based on their family history. Data from the IBIS prevention trials suggest tamoxifen can reduce the incidence of all breast cancer by 38% and oestrogen receptor (ER) positive cancers by 50%. Anastrazole reduced the incidence of all breast cancer by 53% and ER positive cancers by 58%. Despite these promising data, uptake remains low due to concerns regarding side effects, inadequate consultation time and lack of awareness amongst clinicians. In addition, the mechanism of action of preventive therapy is poorly understood – not all women receive the protective effect. In the IBIS I trial, reduction in mammographic density (MD) after one year of tamoxifen was associated with protective effect in a proportion of women. There is an urgent need to determine novel biomarkers indicative of therapeutic response and to develop novel prevention associated with fewer side effects.
Methods: A dedicated breast cancer prevention clinic was established within the family history service at our NHS breast unit, offering half hour appointments to discuss the evidence for available preventive agents and potential side effects. Those keen to proceed were offered the option to provide serial blood samples and an additional mammogram after 6 months, to assess change in MD and identify serum biomarkers indicative of therapeutic response.
Results: This is the first dedicated breast cancer prevention clinic in the UK embedded within routine NHS services. 198 women have attended over the past three years. Uptake of preventive therapy has been substantially higher (52%) than those reported in previous studies (10%). To date, 20 women have enrolled in the biomarker study.
Conclusions: Uptake of preventive therapy can be substantially improved if women are adequately counselled regarding potential risks and benefits. The clinic has provided a valuable resource for future preventive biomarker discovery.

Two Cases of Metaplastic Breast Carcinoma with Osteosarcomatous Differentiation

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We describe two cases of metaplastic breast carcinoma with osteosarcomatous differentiation diagnosed at our Trust.
The first case was that of a 61 year old woman who underwent mastectomy for a large fungating tumour which had replaced most of the breast tissue. On gross pathological examination the tumour was solid, white, with hard areas and areas of necrosis. Histology showed an atypical spindle cell tumour with areas of malignant chondromatous and osteogenic differentiation. There was prominent mitotic activity, with large areas of necrosis and ulceration of the overlying epidermis. The appearance and immunoprofile was of a metaplastic carcinoma with heterologous differentiation (chondromatous and osteogenic). One of the lymph nodes in the axillary clearance showed metastasis from the main tumour.
The second case was that of a 54 year old woman who underwent mastectomy following a biopsy which had been reported to show osteosarcoma. The nipple was depressed overlying a 43mm tumour. The tumour was haemorrhagic and friable upon specimen slicing. Histology showed a metaplastic carcinoma with predominant heterologous differentiation (osteogenic sarcoma) and a minority component of grade 3 invasive ductal carcinoma with associated high grade ductal carcinoma in-situ. No evidence of malignancy was seen in palpable nodes taken from the axilla.
Metaplastic breast carcinoma is a rare and generally aggressive form of breast cancer and both of these patients died within 12 months of diagnosis. Both cases showed osteosarcomatous differentiation. The main differential diagnoses of phyllodes tumour and primary breast sarcoma are discussed alongside important pathological prognostic factors in these types of cases.

A Study of Symptomatic Breast Needle Core Biopsies Categorised as B3 Over a Period of Three Years with Review of Surgical Follow-Up and Comparison with Outcomes from Breast Screening Programme Cases

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Purpose of the study: Breast needle core biopsies within the B3 category represent a heterogeneous group of lesions with a positive predictive value (PPV) for malignancy ranging from 9.8% to 35.1%. Much of the data is drawn from cohorts of screening cases; this study provides insights from symptomatic patients by comparing the range of cases categorised as B3 within our institution with those previous studies and by reviewing the subsequent histology.
Methods: Breast needle core biopsies performed at our institution from 2012 to 2014 were identified from the pathology records. Biopsies that had been reported as B3 were identified by reviewing the reports. Cases reviewed as part of the NHS Breast Screening Programme were excluded. Clinical and radiological indications were recorded from the details provided on the pathology request forms. Follow-up in these cases was recorded by reviewing subsequent histology reports or electronic patient records if there was no further surgical intervention. The slides for the initial needle core biopsies and the subsequent excisions were retrieved from file and reviewed.
Summary of results: B3 cases represented 3.97% of all symptomatic breast needle core biopsy cases. Papillary lesions made up the highest proportion at 46%; these were upgraded to DCIS or invasive carcinoma in 7.9% of cases. Atypical intraductal epithelial proliferations represented 19% of cases but were upgraded to DCIS or invasive carcinoma in 36% of cases. 17% of B3 cases were fibroepithelial proliferations, of which 37% were phyllodes tumours, however, these were rarely malignant. Lobular in situ neoplasia and radial scars made up the remainder of B3 diagnoses.
Conclusions: Our study has shown a proportion of B3 cases (3.97%) and PPV for malignancy (8.75%) at the lower end of the ranges in screening-identified cases (3-9.2%, PPV 9.8-35%) reflecting differing populations and pathology. Atypia was associated with increased likelihood of upgrade to malignancy on excision.

Clinicopathological Characterization of Breast Cancer in Africa: A Tissue Microarray Study of Two African Cohorts

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Introduction: While breast cancer (BC) is a major health issue in Africa the disease, is still poorly understood. We aim to study the presentation and molecular profile of African breast cancer as compared to the European counterpart.
Methods: Two breast cancer cohorts representing North Africa (Egypt, n=84) and West Africa (Nigeria, n=88) were assembled into tissue microarrays. Sections were stained for hormone receptors, Androgen receptor (AR), CK14, Ki67, GATA3, and PDL-1 and scored by two pathologists.
Results: 46.4% of Egyptian and 52.3% of Nigerian patients were under the age of 50. 64.2% of the Egyptian cases showed luminal and 23.5% a triple negative phenotype. 57% Of Nigerian patients were triple negative and only 37.5% of them were of luminal phenotype. PDL-1 showed positive expression in 53.7% of Egyptian and 15% of Nigerian tumours. AR was positive in 38.5% and 47% of Egyptian and Nigerian cohorts respectively. GATA3 was positive in 78.2% of Egyptian and 10.3% of Nigerian cases. 18.7% of Egyptian and 24.3% of Nigerian patients were of the basal phenotype.
Discussion and conclusion: Around half of African breast cancer present at a young age (<50yrs). Compared with the Caucasian BC, Breast cancer in Nigerian was predominantly of the triple-negative phenotype. GATA3 was remarkably low in the Nigerian cohort. The proportions of luminal cancers, as well as AR positive cancers, were low in the two African cohorts. PDL-1 was expressed in tumour cells within a larger proportion of tumours compared with the known Caucasian data. Our data highlight differences in the presentation, immunophenotype and molecular profile between the African and European breast cancer. We are investigating in detail the role of tumour microenvironment and PDL-1 in the African tumours.

Moving to a Digital Pathology Supraregional Germ Cell Tumour Service

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Patients with testicular cancer are managed in supraregional networks serving a population of 2–4 million, seeing at least 100 new patients/year. Patient management includes the review of diagnostic glass slides from local sites for the supraregional MDT. Digital reporting of testis cases was piloted as part of a move to full digitisation of the cellular pathology laboratory at our institution. Feasibility of digital referral from the local centres to the supraregional site centrally was assessed, avoiding the need for postage of slides. Four Philips slide scanners were deployed in 2018 (2 in the central site and 1 in each of two peripheral centres). The service was evaluated as a traditional glass-slide based service in preparation for the switch to digital. A central site validation of digital reporting SOP based on the RCPath guidelines was created. Two specialist germ cell tumour pathologists reported 57 cases on glass slides from 6 sites (benign and malignant). The number of slides ranged from 3–75/case (mean 17), with an average of 13 H&E slides/case. 36 cases had IHC. Mean reporting was 18 minutes/case (range 7–49). The retrospective digital training cases (n=26) were rated on a Likert scale from 1–7 (not at all-very confident). Confidence in digital diagnosis for one pathologist was 4–7 with 23 cases rated 6 or 7. Glass was preferred in 6/26 cases. In 20/26 cases there was no preference between digital and glass diagnosis. Searching for small foci of germ cell neoplasia in-situ was reportedly easier on glass. Prospective validation started with the first digitally reported case of Seminoma in November 2018. The feasibility of running a fully digital supraregional germ cell tumour service across our region shows great promise. Digital reporting of testis cancer cases is feasible and has the potential to improve the availability of slides for supraregional review.

Comparison between Glass and Digital Scans for Maximum Cancer Core Length (MCCL) Measurement and Prostate Cancer Gleason Scoring

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Purpose of study: The use of digital pathology has the potential to improve patient care. We aimed to compare the estimation of MCCL and Gleason sum between glass and digital scans in a cohort of patients at at London hospital.
Methods: 30 patients with Gleason 3+4 (n+15) and 4+3 (n=15) from the PROMIS study were included. A total 192 slides were scanned using NanoZoomer-SQ digital slide scanner. Using NDP.View 2 software each core compromised by cancer was manually measured in mm, if the core was not straight the measurement was added following the shape of the core. For each patient each pathologists provided an overall Gleason sum, blinded to the original glass diagnosis. Wilcoxon matched pairs signed rank test was performed using R.
Summary of results: 426 cores were analysed, the average MCCL was 9.53 mm (5-15) and digital MCCL (dMCCL) was 9.88 (5.01-15.7). In 20 cases the dMCCL was higher than the MCCL ($p = <0.0001$). In 10 cases the MCCL was higher than the dMCCL ($p=0.002$). When taking into account a difference of more than 1 mm between the two measurements 12 were had a higher dMCCL and 7 a higher MCCL. It is important to note that the difference between the two measurements was less than 2 mm in 22 cases (73%), confirming good accuracy for the glass measurement. When estimating the overall Gleason sum per patient, when examining the digital images one patient was downgraded from 4+3 to 3+4 by both pathologists. One patient was downgraded by the more experienced pathologist, and two were upgraded by the less experienced pathologist.
Conclusions: Digital scoring is comparable to glass examination of prostate biopsy specimens. With the possibility of more in depth analysis of digital images and potential reclassification of patients.

Extra-Mammary Paget's Disease of the Penis with Underlying Poorly Differentiated Adenocarcinoma: A Case Report

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Extra-mammary Paget's disease (EMPD) is a rare and slow growing neoplasm and is seen much less often than mammary Paget's disease.1 EMPD presents in regions with a large number of apocrine glands and the ano-genital region is the most common site to be involved. It can be associated with an underlying carcinoma or distant metastases. Patients commonly present with pruritus and a slow growing crusting, scaling, non- healing lesion.2 The symptoms can mimic benign inflammatory skin conditions leading to a misdiagnosis. EMPD is more commonly seen in the elderly with more cases reported in the vulval region of females. Cases involving men are much less common. EMPD in men usually involves the scrotum and involvement of the penis is rare.

We present a case report of an elderly male whose diagnostic biopsy was initially reported as a poorly differentiated squamous cell carcinoma. The patient underwent a penectomy. Histology demonstrated EMPD with characteristic pale cytoplasm and large pleomorphic nuclei with an underlying poorly differentiated adenocarcinoma.

Penile involvement with EMPD and associated poorly differentiated adenocarcinoma is an extremely rare entity with few cases reported in the literature.4 Surgical excision is recommended but EMPD is difficult to manage due to the multifocal nature of the disease.5,6 When associated with invasive malignancy reports suggest there is up to a 46% higher risk of mortality highlighting the need for the timely diagnosis and management.6,7

Concordance and Discordance of Gleason Scores in Prostate Biopsies with Matched Radical Prostatectomy Specimens

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Background: Prostate adenocarcinoma is the most common male cancer in the UK and accounts for 26% of new cancer cases (1). The Gleason Score offers key prognostic information, directs the management of patients with prostatic adenocarcinoma (2). Research has shown that discrepancies exist between the Gleason Score of the preoperative prostate biopsies (PB) and that of radical prostatectomy (RP). Prostatic cancer characteristically shows histologic heterogeneity which contributes to the grading discrepancy between PB and RP (3).
Methods: Data was retrospectively collected for RP cases performed in University College London Hospital (UCLH) from 1st January to 18th March 2019 using Co-Path Database. The Gleason scores of RP and the matched PB were reviewed and compared.
Results: 134 cases of RP were performed at UCLH during the period. In 73 cases (54.5%), the Gleason score was concordant with the preoperative Gleason Score, while 61 cases (45.5%) were discordant with the original biopsy Gleason Score. Of the 54 cases, 50 cases were upgraded and 11 cases were downgraded from the original Gleason Score.
Conclusion: A significant proportion of prostatic adenocarcinoma is under-graded in PB compared to RP, which may impact the patient management and clinical outcome.
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An Unusual Renal Pelvis Tumour

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Large cell neuroendocrine carcinomas (LCNEC) are high grade malignancies which display characteristic morphological features and immunohistochemical evidence of neuroendocrine differentiation. They occur across a range of organ systems but are extremely rare in the upper urinary tract, with very few reported cases in the literature.

We present a case of an 80 year old man who presented with right iliac fossa pain and haematuria. Imaging showed an obstructing mass within the right renal pelvis. Subsequent nephrouretectomy revealed a polypoid renal pelvis tumour composed of sheets of medium cells with scant cytoplasm and elongated, granular nuclei. Marked nuclear pleomorphism was present, with scattered multinucleate giant cells, and the tumour showed abundant mitoses, apoptotic bodies and coagulative necrosis. Focal rosette formation was also identified. Immunohistochemistry revealed evidence of neuroendocrine differentiation, with the tumour cells staining positively for CD56, Chromogranin and Synaptophysin. The proliferation index was measured at >90%. Dot positivity was seen with AE1/3 and CK7. Focal Gata3 positivity was also identified.

These morphological appearances and immunohistochemical profile are consistent with a large cell neuroendocrine carcinoma of the renal pelvis. This diagnosis carries a poor prognosis.

Comparative Study between Immunohistochemical Expression of Erythroblastosis E26 Rearrangment Gene (ERG) and Membrane Associated Guanylate Kinase (MAGI-2) in Prostatic Carcinoma

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Prostate carcinoma (PC) is the commonest non cutaneous cancer in men in the USA. In Egypt, it represents 61.63% of all male genital tract malignancies. The relative lack of specificity and sensitivity of p63 and AMACR in morphologically equivocal cases resulted in significant over and under -diagnosis of PC. Thus it has been important to search for other diagnostic markers with high sensitivity and specificity. The aim of this study is to evaluate the diagnostic and probable prognostic value of immunohistochemical expression of MAGI-2 in comparison to ERG in prostatic adenocarcinoma (PAC). This prospective study included 56 cases of PAC and 29 cases of nodular prostatic hyperplasia (NPH). Results revealed significant difference between NPH and PAC regarding ERG expression ($p< 0.001$). While all NPH cases were negative for ERG, 29 of studies cases of PAC (51.8%) were positive. Thus, the diagnostic power of ERG in PAC revealed 53.6 % sensitivity and 100% specificity. Furthermore, it was noted that ERG expression was statistically significantly related with Gleason grading in PAC ($p=0.005$) and with Ki67 expression ($p<0.001$). Regarding MAGI-2 expression there was a highly statistically significant difference between NPH and PAC ($p<0.001$). While 51 cases (91.1%) of PAC were positive, positive MAGI-2 expression was detected in only 5 cases (17.2%) of NPH. Thus, the diagnostic power of MAGI-2 in PAC revealed 91.1 % sensitivity and 86.2% specificity. Regarding probable prognostic role of MAGI-2, there was no statistically significant relation with any histopatholoic prognostic parameters including Ki67 expression. In conclusion, this study recommend to add both ERG and MAGI-2 to the diagnostic panel of PAC due to different sensitivity and specificity

Clinicopathological Features of Incidental Prostate Cancer in Cystoprostatectomies

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Introduction: Incidental prostatic adenocarcinoma (CaP) in radical cystoprostatectomy specimens is frequently encountered, its incidence ranging from 15 to 54%1,2. Our aim was to determine how many clinically significant incidental CaPs were identified at our centre 2016–2018 to assess whether complete prostate sampling is justified.
Material and methods: Cystoprostatectomies received at our centre 2016–2018 were retrievedand cases with concomitant CaP were analysed fortumour diameter, Gleason grade, stage andmargin and nodal status. Cases with a previous diagnosis of CaP were excluded.
Results: Incidental CaP was identified in 61/135 (45%) cases. Gleason grade was 3+3 in 17 (28%), 3+4 in 32(52%), 4+3 in 6(10%), 4+4 in 2 (3%) and 4+5/5+4 in 4 cases (6%). 48/61 of cases were organ confined (pT1a-pT2c, 79%), 11 cases showed extraprostatic extension (pT3a, 18%) and 2 cases had SV involvement (pT3b, 3%). Margin involvement was present in 4 cases (6%), and one had nodal spread (1.5%).18/61 cases (30%) had features indicative of clinically significant disease, as defined as showing any of the criteria of predominant Gleason pattern 4, pT3a/b status, positive surgical margin or nodal involvement. Additionally, 13/61 further organ confined cases (21%)of Gleason 3+3/3+4 cases had a tumour diameter >10mm.
Conclusion: In our institution a significant proportion of incidental CaPs diagnosed in cystoprostatectomies possess features indicative of clinically significant disease. This supports complete rather than partial sampling of the prostate, previously shown to improve the detection of clinically significant CaP3.The clinicopathological features may however evolve with time due to increasing emphasis on early detection of CaP.

A Study on the Value of Frozen Section Examination of Margins in Penile Resections (2014–2016)

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Introduction: We noticed an increase in the number of penile resection specimens received by our unit. These resections are associated with multiple frozen sections (FS) of margins. We wished to examine the utility of this.
Aim: To determine the caseload associated with FS examination of penile margins, the rate of positivity, false positive and negative rates and comparison with resection specimen and risk of local recurrence.
Method: We assessed our records to identify all penile cancer cases with associated FS examination over a three year period. We compared the FS report with subsequent paraffin sections and local recurrence data for this cohort.
Results: A total of 142 FS on 33 patients were studied (27 in 2014, 25 in 2015 and 90 in 2016 representing 6, 6 and 21 patients respectively) with increased technical and consultant hours noted over the study period. An average of 4 FS were submitted per patient (range 2–6) representing 34% of all uropathology FS in 2014 compared to 86% in 2016. Only one patient had a positive FS (0.7% positive rate) with 100% concordance rate to paraffin blocks. Comparing the results of the FS with subsequent margins in the resection specimen, there were 6 cases with negative FS with an involved margin status of which two developed local recurrence in the follow up period.
Conclusions: Most FS specimens were negative in our cohort. Replacement of random sampling of resection margins with targeted sampling of periurethral/fascia tissue in larger tumours of higher stage is likely to be a more precise and appropriate.

The Matter of Margins: An Audit on the Reporting of Margin Status in Radical Prostatectomies

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Introduction: Positive surgical margins in radical prostatectomy cases are of prognostic significance as well as providing feedback on surgical technique. The Royal College of Pathologists' core data items for the reporting of margin status include margin positivity, the extent of involvement, location and whether involved margins are intraprostatic or extraprostatic. This audit aimed to assess adherence to the core data items in reporting.
Methods: All radical prostatectomy cases for prostate cancer reported between 1st August 2017 and 31st July 2018 were identified and audited on adherence to the following criteria within reports: 1) Margin status; 2) Extent of margin positivity measured; and 3) Location, including whether involvement was intraprostatic or extraprostatic. A standard of 100% for all criteria was used.
Results: 168 radical prostatectomy cases were identified. Margin status was reported in 100% of cases, revealing 64 cases (38.1%) to have positive margins. The extent of margin involvement was measured in 53 cases (82.8%). The location of margin involvement was reported in 100% of cases, whilst intraprostatic margin positivity was reported specifically in 19 cases (29.7%) overall.
Conclusions: The standard was not met for reporting the extent of margin involvement. Most reports omitting this involved basal or multiple margin positivity (72.7%). Intraprostatic margin positivity, which may be reflective of surgical technique, was formally commented upon in only 45.4% of cases with positive radial margins and this is an area which requires further focus within our reports.

An Audit of Renal Tumour Needle Core Biopsy: Single Centre 10 Year Consecutive Case Series

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Purpose: An audit of histological diagnoses, ancillary tests and correlation with final resected diagnoses for a consecutive series of needle core biopsies of renal masses underpinning Scottish Quality Performance Indicator (QPI) for Histological diagnosis.
Methods: All renal tumour needle biopsies were identified by departmental computer search for the period January 2008 to June 2018. Pathology reports were assessed for diagnosis and use of ancillary diagnostic techniques including immunohistochemistry (IHC) and whole chromosome flourescent in situ hybridisation (FISH). Concordance of biopsy diagnosis with final diagnosis was assessed for those cases subsequently undergoing partial or radical nephrectomy.
Results: 220 separate cases were identified, with the following histological diagnosis: 142/220 (65%) biopsies were malignant, 40/220 (18.1%) benign. The remaining 38/220 (17.2%) were not diagnostic. 28.2% of cases (62/220) underwent subsequent surgical resection. The concordance between cases diagnosed as malignant on biopsy which were then resected was 100% (62/62). There were no benign-malignant reversals.
Specific biopsy diagnoses were as follows: 40.4% clear cell RCC; 12.3% oncocytomas; 10% papillary RCC; 4.5% invasive urothelial cell carcinomas; 1.8% RCC not otherwise specified. 3.6% each for Chromophobe RCC, metastatic carcinomas from other sites and angiomyoliypoma. All resected clear cell RCCs were reported with Fuhrman/ISUP grade.
IHC aided diagnosis on 56.4% of cases and FISH was requested on 14.1% of biopsies.
Conclusion: Our single institution experience demonstrates that histology of needle biopsies of renal tumour masses in conjunction with ancillary tests, where appropriate, is an effective diagnostic tool that plays a significant role in informing treatment decisions. The QPI for histology in renal cancer are consistently achieved.

Renal Biopsy Findings in Recurrent POEMS Syndrome

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Glomerular disease is an uncommon manifestation of POEMS (Peripheral neuropathy, Organomegaly, Monoclonal protein and Skin changes) syndrome found in about 4% of cases. We present here the glomerular changes with recurrent POEMS syndrome after apparently successful treatment. A 53 yr old male had previously presented with peripheral neuropathy and was found to have POEMS syndrome. At presentation he was found to have proteinuria, a renal biopsy showed glomeruli with diffuse endothelial swelling. He received an autologous stem cell transplant resulting in complete remission. On routine follow up after 11 years he was found to have developed renal impairment and significant proteinuria, this was investigated by a second renal biopsy. The biopsy showed 20% of glomeruli were globally sclerosed. All other glomeruli were enlarged and showed a diffuse proliferative glomerulonephritis appearance with lobular architecture and both endocapillary and mesangial proliferation. Focal GBM splitting was seen on silver stain. No deposit was identified by IHC or EM. Electron microscopy showed extensive endothelial swelling with luminal occlusion. On further investigation circulating vascular endothelial growth factor (VEGF) was found to be significantly elevated and although no other manifestations of the syndrome were present this was considered evidence of a relapse of his POEMS syndrome. He was subsequently treated with lenalidomide with significant improvement in renal function. Only one previous case of a glomerular relapse of POEMS has been reported, this showed similar renal histology with endotheilal swelling and hyperplasia without either thrombosis or inflammation; this renal pathology is thought to be relatively specific to POEMS and a result of the circulating cytokines, particularly VEGF.

A Comparative Audit of Current and Previous Renal Biopsy Diagnoses

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Primary diagnosis and request form data from May 2017–Nov 2018 of medical native renal biopsies received in a tertiary centre were audited and compared with previous audits from 1990, 2003 and 2011. There were 159 biopsies (M: 95, F: 64), ages range from 1 to 88 (median: 53). All cases had a documented clinical reason for biopsy: proteinuria (nephrotic range and sub-nephrotic) and suspected AKI being the most common. IHC was available in 157, and EM in 150. The primary diagnoses were: IgA nephropathy: 25 cases (16%); FSGS: 25 cases (16%); membranous GN: 12 cases (8%); minimum change and immune complex GN: 10 cases (6%) each; and vasculitis: 8 cases (5%). A range of less common diagnoses were recorded including acute interstitial nephritis, lupus, diabetes, and amyloid. No case was reported as normal or failed in this period. The distribution of diagnoses showed differences to the distributions seen in previous similar audits undertaken in the years 1990, 2003 and 2011. There was a reduction in the incidence of diabetic nephropathy, thin membrane nephropathy and IgA nephropathy; this reduction likely reflects changes in the criteria for biopsy. More puzzling was a reduction in the incidence of vasculitis and membranous GN as clinical suspicion for these diseases are strong indications for biopsy. It is possible that this represents true changes in the incidence of these disease. In contrast there was increase in the incidence of FSGS; the majority of the FSGS cases were not associated with nephrotic syndrome and while several cases were biopsies of suspected relapsed vasculitis this does not fully explain this increase. In summary the audit demonstrates consistent and complete examination of medical renal biopsies and a wide range of primary diagnoses, but with changes in the frequency of some diagnoses over time that require explanation.

Pleomorphic Rhabdomyosarcoma of the Uterus: A Molecular and Immunohistochemical Analysis of a Rare but Aggressive Gynaecological Malignancy

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Purpose of study: Pleomorphic rhabdomyosarcoma (PRMS) of the uterus is an aggressive sarcoma associated with poor prognosis. Cases are rare and treatment regimens are not well established. The aim of this study was to identify cases of PRMS at our hospital and perform molecular and immunohistochemical tumour analysis.
Patients and methods: Two cases of PRMS of the uterus were identified at our institution over a ten year period (2009–2019). The women were aged 84 and 68 years old respectively. The patients underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy. We reviewed the clinical data and histology of both cases. An immunohistochemical panel was performed on selected tumour blocks. Tumour DNA was sequenced using a targeted next generation sequencing (NGS) panel consisting of 50 genes.
Summary of results: Both tumours were positive for desmin and myogenin. They were negative for CD117 and PDL1. Analysis of the tumour immune response demonstrated a dense tumour infiltration by CD68+ macrophages. A pauci-immune response from CD4 and CD8+ T cells was noted. Somatic DNA sequencing failed in one case due to excessive fixation. In the other case, two mutations were identified: a PIK3CA p.(His1047Arg) activating mutation and a TP53 p.(Arg248Gly) loss of function mutation.
Conclusions: We present the first evidence of an actionable mutation in uterine PRMS. P13K inhibitors are currently a focus in multiple clinical trials for other malignancies. Activation of the P13K/AKT/mTOR pathway by mutations in the PIK3CA gene has been documented in other subtypes of rhabdomyosarcoma at sites outside of the gynaecological tract; blockade of this pathway has revealed promising evidence for further therapeutic strategies. Furthermore, this study shows for the first time that uterine PRMS is a macrophage-rich tumour. If this correlates with poor survival, as in many other cancer-types, the therapeutic potential of macrophage inhibitors could be explored.

Exceedingly Rare Pure Large Cell Neuroendocrine Carcinoma of the Endometrium: Report of Two Cases

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Introduction: Less than 1% of endometrial carcinomas are neuroendocrine carcinomas (NECs). Most endometrial NECs are small cell neuroendocrine carcinomas, with ~90 cases reported to date. Endometrial large cell neuroendocrine carcinoma (LCNEC) is rare with <20 cases reported to date. LCNEC has a poor prognosis with early hematogenous/ lymphogenous metastasis.
Case presentation: We present two cases of endometrial LCNEC.
Case 1: A 64 year old hospital secretary presented with postmenopausal bleeding. Ultrasound revealed a 3 x 4 cm intrauterine mass. Hysteroscopy confirmed a fundic mass with necrotic edges. The biopsy and subsequent total abdominal hysterectomy, bilateral salpingo-oopherectomy (TAHBSO) and lymphadenectomy revealed a 60 mm FIGO stage 2 endometrial LCNEC with extensive lymphovascular space invasion.
Case 2: An 85 year old lady with Alzheimer's disease presented with postmenopausal bleeding. Transvaginal ultrasound suggested an endometrial tumour invading >50% of the myometrium. MRI pelvis revealed a grossly enlarged uterus measuring 17x 10cm with a distended endometrial cavity containing a large and poorly enhancing soft tissue mass. The patient underwent a hysteroscopic biopsy followed by TAHBSO and omental biopsy. The histology showed a 150 mm FIGO stage 2 endometrial LCNEC with extensive lymphovascular space invasion.
Discussion: Pure endometrial LCNEC is rare and diagnostically challenging. As most endometrial NECs are admixed with other endometrial carcinoma subtypes; extensive sampling is essential to confirm the rare pure NECs. The differential diagnosis includes a solid component of endometrioid carcinoma, undifferentiated carcinoma, a sarcomatoid component of carcinosarcoma or primitive neuroectodermal tumour . Pathologists should be aware of the histological features of these rare tumours and the potential pitfalls with immunohistochemistry.

Leydig Cell Tumour of the Testis and Ovary with Rare Metaplastic Changes: Case Comparison and Review of the Literature

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Leydig cell tumours are a type of sex chord stromal tumour found in the testis; however they can also occur in the ovary. Leydig cell tumours represent 1–3% of all testicular tumours but comprise less than 0.1% of ovarian tumours. In men, they arise at a broad age range between 20–70 years with some tumours occurring in prepubertal children. In contrast, Leydig cell tumours of the ovary occur mostly in post-menopausal women with an average age of 60. In both sexes these tumours commonly present as an incidental finding, although they can produce endocrine symptoms such as feminisation or virilisation in males and virilisation in females. Macroscopically they tend to be small and unilateral but 10% of testicular Leydig cell tumours can be bilateral. Importantly 10% of Leydig cell tumours of the testis are malignant compared to their ovarian counterparts which are almost entirely benign. Microscopically they are typically uniform and composed of sheets of polygonal lipid laden cells but unusual architectural features and metaplastic differentiation such as ossification and adipose metaplasia can be seen. These metaplastic changes are rare and have been previously described in a small case series of testicular Leydig cell tumours but not in Leydig cell tumours of the ovary. We present two cases of a Leydig cell tumour, one arising in the testis of a 50 year old man and the other found incidentally in the ovary of a 70 year old woman. Both cases show a benign Leydig cell tumour with areas of ossification and adipose differentiation. We compare and discuss the histological appearances and diagnostic challenges associated with these unusual features. Critically we describe these changes for the first time in an ovarian Leydig cell tumour and explore the possible pathogenesis and diagnostic implications.

Rare Primary Leiomyosarcoma of the Uterine Cervix: 4 Cases and Review of the Literature

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Case report: A 65 year old woman presented with a 7.2 cm cervical mass. At macroscopic examination the mass was based in the cervix with no involvement of the uterine corpus. Microscopically, the lesion consisted of atypical spindled cells with necrosis and a high mitotic index (40/10HPF). Immunohistochemically the tumour showed expression for smooth muscle markers, confirming the diagnosis of a cervical leiomyosarcoma. Retrospectively, we only found 4 cases (including the current case) of primary cervical leiomyosarcoma over the last 10 years in our institute. The ages at diagnosis were between 23 and 65 years and one patient was pregnant. The tumour diameters were between 5 and 8 cm. The oestrogen receptor was negative in 3 cases while one case showed weak staining. The progesterone receptor was negative in 2 cases and strongly positive in 2 cases. We have follow up data for 3 patients all of which were treated with radical hysterectomy without lymph node dissection. One patient had local recurrence after 2 years and died 3 years after diagnosis. Two patients are disease free with follow up of 8 years and 2 months respectively.
Discussion: With these 4 cases we would like to draw attention to this rare entity. Primary cervical sarcomas account for less than 1 % of all tumours of the cervix. Cervical Leiomyosarcoma represents 0.21% among all invasive tumours of the uterine cervix. A leiomyosarcoma of the uterine corpus invading in the cervix must be excluded, since this is much more frequent. Rare carcinosarcoma of the cervix should also be excluded by thorough sampling. Leiomyosarcomas of the cervix have a worse prognosis than carcinomas and are therefore important to recognise. Most patients are in their 4th to 6th decade. Due to the rarity of the disease, therapeutic options are extrapolated from uterine leiomyosarcomas but are not well investigated. In view of the rare instances of the involvement of lymph nodes, routine pelvic lymphadenectomy is not recommended.

The Association of Human Papilloma Virus Subtypes with Lymph Node Metastases and Recurrence of Vulval Squamous Cell Carcinoma

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The incidence of global vulval squamous cell carcinoma (VSCC) is 2.5 in 100,000 women per year and it represents between 2–5% of all gynaecological cancers. The incidence of VSCC is rising, specifically in younger women and this has been attributed to rising Human Papilloma Virus (HPV) infection rates. The disease is associated with high morbidity including lymphoedema, incontinence and psychosocial disability. Once the disease has metastasised to the lymph nodes, the prognosis is dismal. In this study we will identify the subtypes of HPV that drive VSCC and correlate them with clinical data including disease recurrence and lymph node metastases. We identified 29 cases of VSCC from our Co-path database at UCLH from 2015 to 2016. We collated the clinical data and diagnostic histopathology reports. All H&E slides were reviewed and an appropriate FFPE block of tumour per case was selected. These were sent to our diagnostic laboratory for p16 immunohistochemistry and for HPV genotyping by PCR array (Zytovision visionarray chip). There were 40 HPV genotypes assessed (12 high risk, 11 probable high risk and 17 low risk subtypes). 31% of these patients are now deceased (9/29) and 89% of the deceased patients had pelvic lymph node metastases (8/9). 7/29 patients experienced one or more disease recurrences post-surgery. In total, 58% of patients had lymph node metastases (17/29), 28% had negative lymph nodes (8/29) and 14% of patients did not have lymph nodes sampled (4/29). The results of the HPV genotyping of each tumour will provide us with important information about HPV driven VSCC and the subtypes that may confer a worse prognosis. A significant number of patients had lymph node metastases. Most patients without lymph node metastases are still alive after 3 to 4 years. This study will improve our understanding of the pathogenesis of HPV-driven VSCC by identifying HPV subtypes associated with metastasis and progression.

Primary Malignant Melanoma of the Female Genital Tract: An Audit of Histopathology Reporting

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Aims/objectives: Primary malignant melanoma of the female genital tract (FGT; vulva, vagina or cervix) is rare, and carries a poor prognosis compared to cutaneous melanoma. Histological prognostic factors are not defined and validated for these tumours. In the absence of published reporting guidelines for FGT melanoma, we assumed that the Royal College of Pathologists' cutaneous melanoma dataset would be used to report them. We performed this audit to investigate whether core dataset items were being reported at our institution.
Methods: All cases of malignant melanoma occurring in the FGT since 2007 at our tertiary referral hospital were identified via a text search of the pathology reporting database (CoPath). Primary FGT origin was confirmed via electronic clinical notes. Excision/resection reports were reviewed, and core dataset items were recorded as present/absent from the report.
Results: 31 FGT melanomas were identified: 20 vulval, and 11 vaginal or cervical. Mean age at diagnosis was 64. 45% of patients were Caucasian. 25% had a history of other previous malignancy, including one BRCA2 mutation carrier. Excision or resection specimen reports were available for 25 cases. Dataset parameters reported for these cases included: tumour subtype (56%), Breslow thickness (91%), ulceration (68%), mitotic index (23%), lymphovascular invasion (58%), regression (23%), stage (32%), completeness of excision (92%).
Conclusions: Key melanoma dataset items are often absent in reports in primary FGT melanomas. In view of the rarity of these tumours and the paucity of known prognostic indicators, we recommend routine use of the RCPath dataset for cutaneous melanoma to increase the knowlege base in this area. Reporting could be improved by the use of proformas and specialist dermatopathology input.

Distribution of Leiomyosarcomas in the Female Genital Tract: A 10 year Retrospective Study

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Introduction: Of all sarcomas in the female genital tract, leiomyosarcoma of the uterine corpus has the highest incidence (54% of all sarcomas). The myometrium is the commonest location because of the abundance of smooth muscle. Leiomyosarcomas at other locations in the female genital tract are much less frequent and are thought to arise from vascular smooth muscle bundles. With this study we aim to investigate the distribution of leiomyosarcomas in the female genital tract.
Methods: We performed a retrospective search over the last 10 years in the archive of our tertiary hospital. All patients with a biopsy or resection from a primary tumour were recorded and when there was a biopsy or resection of a metastasis, the location of the primary tumour was noted.
Results: In total 76 patients were diagnosed with a leiomyosarcoma in the female genital tract over the last 10 years. Of these, 64 were primary uterine leiomyosarcoma. The other locations of primary leiomyosarcoma were vagina (5), cervix (4), vulva (2) and fallopian tube (1).
Discussion: Although leiomyosarcoma are most frequent in the corpus uteri, more than 15% of our cases had another primary origin in the female genital tract. These included all other organs, except the ovaries. Ovarian leiomyosarcomas are described in the literature but are very rare. This distribution appears to have remained stable over time as our data is concordant with older literature that showed that 86% of the gynaecologic leiomyosarcomas are uterine. With this report we want to emphasise that leiomyosarcomas have a more heterogeneous site distribution than mostly thought and that a smooth muscle neoplasm must be considered in every spindle cell lesion of the female genital tract.

A Rare Diagnosis after Investigation for Infertility

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We present this case of a 38 year old female patient who underwent an exploratory laparoscopy for investigation of infertility and abdominal pain. At the time of surgery multiple solid white nodules were noted and biopsies were obtained from the gynaecological tract, bladder and peritoneum. Histology showed that all the biopsies comprised a papillary tumour with fibrovascular cores lined by pleomorphic epitheloid tumour cells. Immunohistochemistry was performed and the tumour cells were positive for WT1, Calretinin, podoplanin and CK5/6, confirming mesothelial origin. The tumour cells were negative for Pax-8, p53, p16, ER and PR. The clinical, histological and immunohistochemical features were consistent with a diagnosis of primary peritoneal mesothelioma. Primary peritoneal mesothelioma is a rare but aggressive malignancy, which comprises only a small proportion of all mesotheliomas diagnosed. It is poorly described and the knowledge of its natural history is very limited. Only 20% to 33% of all mesotheliomas arise from the peritoneum itself; the pleura is the most common site of origin. Other locations include tunica vaginalis testis, and pericardium. This tumour is most frequently seen in middle aged adults but can present at any age and usually has a male preponderance. Primary peritoneal mesothelioma is associated with asbestos exposure in approximately half of cases. A reported association with simian virus (SV) 40 remains controversial. This tumour tends to spread throughout the peritoneal cavity, producing a diffuse form of the disease. Treatment options include debulking surgery and chemoradiotherapy. The main histopathological differential diagnosis lies between metastatic carcinoma and reactive mesothelial hyperplasia.

Phyllodes Tumour of the Vulva: A Rare Case Report

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Introduction: Phyllodes tumours of the vulva are uncommon neoplasms with a leaf like architecture and biphasic component histologically. This tumour shares morphological similarities with phyllodes tumour occuring in the breast supported by the immunohistochemical expression of breast markers, oestrogen and progesterone receptors.
Case Report: A 64 year old female presented with lump on her right vulva for a duration of 3–4 months. On examination a 5cm exophytic lump was seen but no lymph nodes were palpable bilaterally. The findings were concerning and a fungating vulval lesion was in the differential diagnosis. A biopsy showed pseudopolypoid fibroconnective tissue stroma covered by epithelium displaying variable appearance ranging from a double epithelial layer to areas with more complex micropapillary tufting with cross-linking, and squamous epithelium. The lesion was subsequently excised and histology showed a biphasic tumour with an epithelial and stromal component. The epithelial component comprised columnar cells with areas of papillary tufting. The stromal component was collagenous and hypocellular with admixed spindle stromal cells lacking cytological atypia. Mitotic figures were scarce and the lesion appeared excised. Immunohistochemistry on both specimens showed positive staining with GATA-3, ER, PR and Vimentin in the epithelial component. SMA, CK5/6 and S100 stained the myoepithelial cells but showed negative staining in the stromal component. The diagnosis of a benign phyllodes tumour was made.
Discussion: Clinically, these tumours are unilateral, solitary, pain less and located in the labia majora. The management is surgical excision with clear margins. Recurrence is rare and no record of metastasis has been reported. In conclusion this rare tumour should be considered in the differential diagnosis for women presenting with a slow growing vulval mass.

Cystic Endosalpingiosis-Associated Clear Cell Carcinoma Arising in a Caesarian Section Scar

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A 48 year old woman was admitted for investigation of a longstanding 3.5cm fungating abdominal skin lesion, which was unresponsive to antibiotics. Past medical history includes laparoscopic investigation for endometriosis and Caesarean section. Ultrasound imaging showed a lobulated soft tissue mass in the right iliac fossa region, extending from ulcerated skin to deep subcutaneous tissue. Skin punch biopsy demonstrated multiple cysts lined by simple columnar to cuboidal epithelium. These cells were positive for CK7, ER, CA-125 and BerEP4, with negative staining including calretinin, WT1, CK20, CK5, p63, TTF1 and CD10 (with patchy stromal staining). There was no atypia, necrosis or mitoses. Consensus diagnosis was of a 'mullerianosis', most in keeping with cystic endosalpingiosis. Abdominal CT imaging identified bilateral inguinal lymphadenopathy. Pelvic organs were normal. Due to the lesion size and associated lymphadenopathy, she underwent excision of the lesion with lymphadenectomy. Macroscopically, there was residual Caesarean section scar abutting the 16 x 12 x 11cm lesion, which had cystic, solid and papillary components. Histology showed clear cell carcinoma, confirmed by positive staining for CK7, CA125 and napsin A, with negative staining for ER, WT1, p16 and p53 (wild type). Staining was repeated on areas histologically similar to the biopsy to confirm the cystic endosalpingiosis component. Clear cell metastases were present in multiple local lymph nodes. Ongoing active treatment options are under consideration.

Total Processing of the Omentum to Identify Microscopic Implants in Patients with Serous Borderline Ovarian Tumours

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Aims: Serous Borderline Ovarian Tumours (SBOTs) may form extra-ovarian deposits known as implants, commonly in the omentum. Thorough omental sampling to detect microscopic implants is crucial for staging SBOTs. However, there is little information for a specific sampling threshold that must be reached to detect implants. We aimed to determine if total omental sampling is necessary to detect microscopic SBOT implants.
Methods: The trust histology database was searched for pathology reports of patients with SBOTs between 2008 and 2019. Completeness of omental sampling, the number of omentum blocks processed, and the detection of implants was noted.
Results: Of 69 cases with total omental sampling, 19 had microscopic implants, compared to 17 in 30 cases that did not undergo total omental sampling. In 23 cases where the omentum was incompletely sampled when initially received, no implants were found and the rest of the omentum was processed. In 3 such cases, implants were found in the extra blocks. On average, 1 further block was taken in cases that contained an implant compared with those that did not contain implants. Results were compared to the recommendation of 10 blocks for a grossly normal omentum. Of 6 cases in which exactly 10 blocks were initially taken with tissue remaining, 2 cases contained no implants and in one of these cases implants were found in further blocks.
Conclusions: Sampling 10 blocks of grossly normal omentum is insufficient to avoid missing implants. Complete sampling of the omentum is recommended to accurately stage SBOTs.

An Unusual Complication of Tension Free Trans Vaginal Tape

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Objectives: We report an unusual complication of Tension-Free Vaginal Tape (TVT) use for posterior vaginal wall prolapse. Histopathologists are not used to seeing complications of the use of TVT in specimens sent to them as these specimens are usually received at certain centres in the UK. We feel that an awareness of the rather serious possible complications that may ensue following the use of TVTs should be known by histopathologists so that there is no confusion with other primary gastro-intestinal pathology. Thorough examination and documentation of these specimens with clinico-pathological correlation is required.
Method: 64 year old female, underwent posterior vaginal wall prolapse repair with mesh and sacrospinious fixation in 2009. Following which, the patient became clinically symptomatic with vaginal and anal pain and dyspareunia. The Magnetic Resonance Imaging revealed migration of the mesh which had launched in to the posterior vaginal wall close to rectum. This was partially removed under emergency colorectal operation in 2015. A year later, she suffered intermittent episodes of systemic sepsis. The 3D ultrasound scan located residual mesh between the lower posterior vaginal wall and the rectum. This resulted in a rectovaginal fistula. She required an ultra-low anterior resection to excise the mesh and fistula. Gross examination of the bowel revealed the mesh in situ within the bowel wall and histological confirmation of microscopic erosion of the mesh into the intestinal mucosa was confirmed. Recto-vaginal fistula was not obvious macroscopically.
Conclusion: Complications of Tension-Free Vaginal Mesh have been associated with medicolegal issues and there are national enquiries about safety of this procedure. We have described here a rare but serious complication of bowel injury following the use of TVT, and feel it is important for the pathologists to be aware of its complication so that appropriate examination and documentation can occur.

An Audit of Atypical Complex Hyperplasia Reporting and Management

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Background: This audit was carried out to determine the proportion of women diagnosed with atypical complex hyperplasia (ACH) who undergo hysterectomy as recommended by the RCOG/BSGE Joint Guideline "Management of Endometrial Hyperplasia". The degree of histological agreement between the initial biopsy and hysterectomy specimen was also investigated.
Method: This was a retrospective selection of all cases for a 14 month period between 01/01/17 and 01/03/18. We reviewed histology reports on the laboratory information system and recorded the biopsy and hysterectomy specimen diagnoses.
Results: 39 cases of ACH were identified during the search period. Of these, 34 patients underwent hysterectomies as per the guidelines. The 5 patients who did not undergo hysterectomies had medical reasons. 16 patients were found to have endometrial cancer after undergoing a hysterectomy. The compliance between the biopsy and hysterectomy diagnosis was 82% (28/39). This figure includes cases whereby the initial biopsy diagnosis was ACH, and in the hysterectomy specimen a diagnosis of at least ACH was present. 15% (6/39) of hysterectomies did not show features of ACH.
Conclusion: The results of this audit have demonstrated that we are meeting the recommended targets outlined by the RCOG/BSGE with the exception of patients who are not medically fit for surgery. The cases showing a lack of correlation between the biopsy and resection specimen were re-reviewed. All six cases had thorough sampling of the endometrium. Reasons for non-compliance included poorly preserved endometrium, progesterone effect in patients started on progesterone therapy, and cases where ACH was identified within a polyp. This audit has highlighted the importance of adequate tissue fixation to enable accurate histological assessment. In addition the use of progesterone therapies appears to be having a therapeutic effect on cases with ACH as demonstrated in 3 cases from our cohort where no residual ACH was identified.

Oligometastasis of Lobular Breast Carcinoma to the Uterine Cervix: A Case Report

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A case report of lobular carcinoma of the breast, metastatic to the uterine cervix, and detected in a routine cervical smear test. A 56 year old female underwent a routine cervical smear. The smear contained groups and sheets of dyscohesive, round to oval cells with pleomorphic, eccentric nuclei and granular cytoplasm, some with targetoid intracytoplasmic inclusions. Abnormal mitoses were noted and tumour diathesis was present in the background. A review of the medical records revealed a history of bilateral breast carcinoma, including a grade 2 lobular carcinoma of the right breast. Histology from the left breast tumour was not available for review. A diagnosis of non-cervical adenocarcinoma was made on the smear with a comment suggesting lobular carcinoma. A tissue biopsy from the cervix was obtained, and a diagnosis of metastatic lobular breast carcinoma was confirmed morphologically and immunohistochemically. Imaging of the chest, abdomen and pelvis revealed no evidence of further metastases. Metastases to the female genital tract from extragenital primary tumours are uncommon. The most common site for genital tract metastases is the ovary. The uterus, especially the uterine cervix, is rarely involved by metastatic tumours. In extragenital tumours metastatic to the female genital tract, the breast is the second most common primary site, behind the gastrointestinal tract. Invasive lobular carcinoma of the breast is renowned for exhibiting unusual metastatic patterns. Invasive lobular carcinoma spreads more frequently to the genital tract than invasive ductal carcinoma. Correct diagnosis is important as management of primary genital tract malignancy and metastatic breast carcinoma differ dramatically. It is therefore important for the pathologist to consider metastatic carcinoma in the differential diagnosis of a cervical mass or abnormal cervical smear.

A Retrospective Analysis of Negative Large Loop Excisions of the Transformation Zone (LLETZs)

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Purpose of the study: The increased risk of preterm birth, mid-trimester miscarriage and ectopic pregnancy following large loop excision of the transformation zone (LLETZ) makes minimising unnecessary procedures critical. This audit quantified and confirmed the histological status of negative LLETZs and determined the indication for, and management pathway to, excision.
Methods: Retrospective analysis of LLETZ specimens (n=1146) from 2016 across two Yorkshire centres identified 81 negative cases which were compared with a randomly selected, size-matched positive comparator group. The indication, punch biopsy dimensions, biopsy-to-LLETZ time interval and the number of levels examined were analysed.
Summary of results: The negative LLETZ rate was 7%. Indications for LLETZ fitted into three categories: see-and-treat at colposcopy following screening (30 vs. 63% in the negative and positive LLETZ groups, respectively), treat despite negative biopsy (15 vs. 1%) and treat following positive biopsy (55 vs. 36%). Mean pre-LLETZ punch biopsy volume was smaller in the positive LLETZ cohort (64.3 vs. 108.1mm3). More negative LLETZs (79%) had multiple levels cut than their positive counterparts (62%). There was no significant difference in biopsy-to-LLETZ time interval across positive and negative LLETZ groups.
Conclusions: These findings suggest that the larger biopsies taken in the negative group have excised the lesions, in essence a therapeutic biopsy. The finding of a negative LLETZ following a positive biopsy adds no clinical value, as the management will be test of cure regardless. This raises the question of whether further levels should be routinely cut and/or tissue reorientated on every block for negative LLETZs, or whether they need multidisciplinary team discussion, as this will not change management. The results also suggest that colposcopists may be more confident in their clinical impression in the positive LLETZ group, with fewer/smaller biopsies being taken.

Risk of Vulval Squamous Cell Carcinoma Amongst Patients with Lichen Sclerosus

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Purpose of study: Vulval Lichen Sclerosus (VLS) is an inflammatory condition of unclear aetiology charaterised by the development of itchy plaques, scarring and local histoarchitectural destruction. Of particular concern is the allied risk of vulval squamous cell carcinoma (VSCC) arising from VLS. Although this risk has been approximated to circa 5%, there is a paucity of large scale studies with appropriate follow-up to corroborate this figure. The aim of this study was therefore to determine the risk of malignant transformation in a large retrospective cohort of patient with VLS.
Method: This study examined the clinical records of 762 women suffering from VLS across St James's University Hospital, Leeds, and Bradford Royal Infirmary across the period spanning 1993 to the present day. Data collected included incidence of VSCC and recurrence rate.
Summary of results: Eighty-eight (11.5%) women with VLS developed VSCC. Of these, 48 (55%) developed a recurrence post intended curative treatment.
Conclusions: These figures markedly exceed current published data relating to malignant transformation rates of women with VLS; these range from <1-5%. Recurrence rates were also higher in our study compared to published data (12–50%). These findings highlight that current figures relating to the malignant transformation of VLS are likely to significantly underestimate true risk. It also underscores for clinicians to manage VLS aggressively in order to prevent the development of VSCC. Further follow-on work will assess the impact of stage, grade and age at diagnosis as well as human papillomavirus status in our current population in order to determine their impact on VLS transformation to VSCC.

Adenocarcinomatous Transformation of a Retroperitoneal Teratoma Mimicking an Adrenal Incidentaloma in an Adult Female: A Case Report and Literature Review

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Retroperitoneal teratomas are extremely rare in adults and are typically benign tumours. We describe a case of a 48-year-old lady who initially presented with abdominal discomfort 18 years ago and was found to have a large right-sided supra-renal mass suspected to be an adrenal lesion. Following initial biochemical and imaging studies a diagnosis of non-functioning adrenal adenoma was made and she was managed conservatively. After being lost to follow up and remaining clinically well for many years, she once again presented with worsening abdominal pain. A right adrenalectomy was performed and the histology surprisingly confirmed a mature cystic teratoma with malignant transformation into a moderately-differentiated intestinal-type adenocarcinoma as well as having a focus of carcinoid tumour. She was admitted shortly afterwards with widespread metastatic disease and sadly passed away before any treatment could be instituted. Only a handful of cases of primary retroperitoneal teratomas have described adenocarcinomatous transformation. A carcinoid tumour within a teratoma is even less common and typically does not manifest systemic features. This case underlines the broad differential diagnosis of an adrenal mass and highlights several unusual aspects of a teratoma. We present the current literature and discuss the appropriate investigations, management and challenges involved in such rare cases.

Platform-Independent Prediction of Malignant Transformation Within Ten Years of Confirmed Oral Epithelial Dysplasia Using Image Analysis

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Objectives: Oral Squamous Cell Carcinoma (OSCC) is one of the more frequently diagnosed cancers worldwide. Some cases of OSCC are preceded by changes in the oral epithelium known as dysplasia. Reported transformation from oral epithelial dysplasia (OED) into malignancy ranges from 3.2-50.0%. Currently there is no reliable method of predicting which lesions progress into malignancy and which don't. The overall aim was to test CD3 and CD8 as potential biomarkers for predicting malignant transformation which was assessed by correlation of CD3 and CD8 expression with progression of OED into OSCC. An additional objective of this study was to compare four image analysis approaches (Qupath, Halo, Visiopharm and Definiens) for quantifying levels of CD3 and CD8 in OED in order to determine inter-platform reproducibility.
Methods: 49 cases of OED with at least two biopsies from the same oral site and with ten years follow up were subcategorised into two groups; (a) patients whose dysplasia progressed into malignancy within ten years and (b) patients whose dysplasia remained as dysplasia within ten years. The levels of CD3 and CD8 were quantified in the two groups of patients using four different image analysis platforms: Halo, Qupath, Visiopharm and Definiens. The inter-platform reproducibility of the test was also observed to compare the four image analysis software's utilised in this study.
Results: Levels of CD3 in the two subgroups of patients demonstrated no significant differences between the two patient groups ($p>0.05$). There was a significant difference noted in the level of CD8 between the two subgroups of patients ($p<0.05$). There was strong correlation of the results produced by the four software ($R>0.7$).
Conclusions: CD8 was confirmed as a potential biomarker for predicting the malignant transformation of OED. The inter-platform reproducibility suggests that the analysis of the biomarkers tested in this study should be similar when performed by any platform.

Identification of Novel Copy Number Alterations in Ameloblastoma and Ameloblastic Carcinoma from Nigeria

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Purpose of study: Ameloblastoma is a benign odontogenic neoplasm, characterized by local invasiveness, facial deformity, tooth displacement, a high rate of recurrence, and malignant transformation. It accounts for 63% of odontogenic tumours in Nigeria. Recently, studies in the genomic landscape of ameloblastoma have identified a number of consistent alterations that may be useful for therapeutic intervention. To date, no whole genome survey of ameloblastoma and ameloblastic carcinoma has been published.
Methods: DNA was extracted from RNALater stored tissue using the DNeasy Tissue Kit (QIAGEN), from a cohort of ten ameloblastoma and three ameloblastic carcinoma from UCH, Ibadan, Nigeria. Whole genome analysis was performed using the Oncoscan FFPE Assay Kit (Affymetrix). Data was analysed using Nexus Express for Oncoscan 17.0 and Somatic Mutation Viewer 1.0.1.
Results: Ameloblastoma (n=10) showed a mean genome change of 9.7%, with a mean of 88.7 copy number (CN) aberrations and 7.5% of loss of heterozygosity (LOH), whereas the ameloblastic carcinomas (n=3) had a mean genome change of 6.8% with a mean of 87.3 copy number (CN) aberrations and 3.6% of loss of heterozygosity (LOH). All tumours (benign and malignant) showed CN gain at 8q23.3, affecting the CSMD3 gene. Other commonly affected regions included LOH at 1p34.2-p34.1 and 2q11.2, among others. Ameloblastoma and ameloblastic carcinomas shared somatic mutations in BRAFV600E, EGFR, KRAS and PTEN genes. One ameloblastoma showed a mutation in TP53 and two (66.7%) ameloblastic carcinomas showed a mutation in the PIK3CA gene, which was not observed in the ameloblastoma cohort.
Conclusions: Ameloblastoma and ameloblastic carcinoma do not show extensive genome changes indicative of genomic instability. We have identified novel areas of CN gain and LOH that require further investigation. The mutational profile of these lesions is similar to that reported in the literature.
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Hypoxia and HPV Status in Oropharyngeal Squamous Cell Carcinoma

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Hypoxia attributable to disorganised vascularisation variously features in the microenvironment of solid tumours and adversely affects response to treatment and prognosis. The present investigation aims at exploring whether tumour-cell phenotypes related to micro-environmental oxygen levels are influenced by the integrated human papilloma virus (HPV) infection in oropharyngeal squamous cell carcinoma (OPSCC). Microarrays or full sections from formalin-fixed, paraffin-embedded tissues of 41 HPV(+) and 34 HPV(-) OPSCCs were examined by means of immunohistochemistry for 'anaerobic' phenotypes (hypoxia inducible factor 1 alpha, HIF1α), glucose transporter 1 (GLUT1) and (on a smaller cohort) monocarboxylate transporter 4 (MCT4); mitochondria (TOMM20); and 'angiogenic' phenotypes (vascular endothelial growth factor, VEGF). Membranous expression of MCT4 was seen in the front of 40/41 and 26/34 HPV(+) and HPV(-) tumours, respectively ($p = 0.023$). Nuclear expression of HIF1α was seen in the centre of tumour cell aggregates in both front and core of 4/10 HPV(+) and 8/10 HPV(-) tumours ($p = 0.08$). Membranous GLUT1, cytoplasmic granular TOMM20 and cytoplasmic diffuse VEGF expression were seen throughout the tumour parenchyma, independently of the HPV status (10/10 and 10/10). The results suggest that while the tumour cells in HPV(+) and HPV(-) OPSCC show similar mitochondrial load and transport glucose, their 'anaerobic' profile differs. Tumour cells in HPV(+) tumours more frequently release lactate via expression of MCT4, whereas hypoxia inducible transcription factors are more commonly synthesised by HPV(-) tumour cells. 'Angiogenic' phenotypes appear similar in both groups of tumours and not directly related to those differences.

Spatial Relationships Between Immune Infiltrate and Tumour Buds Improves Prognostic Accuracy in Stage II Colorectal Cancer

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Purpose of the study: Within the tumour microenvironment, cancer cells coexist and interact with the heterotypic immune system. Tumour budding and the immune infiltrate are established prognostic factors in stage II colorectal cancer (CRC), though the importance of their spatial interaction is less studied. The objectives of this research were to determine the prognostic value of the dynamic interplay between tumour buds (TBs) and infiltrating immune cells.
Methods: Multiplexed immunofluorescence for TBs, CD3+, CD8+ lymphocytes and CD68+, CD163+ macrophages was performed across two sequential whole tissue slides (n=232). Automated image and spatial analysis was applied to quantify the distinct cell populations and their spatial interactions. Machine learning was used for the development of a prognostic risk model.
Summary of results: TB (p=0.001) and lymphocytic density (p<0.001) were found to be significantly correlated with disease-specific survival. However, a novel prognostic model which also incorporated their spatial relationship was shown to better stratify stage II CRC patients at high risk for disease-specific death (p<0.001) compared with the clinical gold standard of pT stage (p=0.003). The model was developed using data from 114 patients and validated in two independent cohorts (cohort 1: n=56 and cohort 2: n=62). Furthermore, a low ratio of CD68+/CD163+ cells at the tumour core was associated with better survival (p<0.001). A prognostic model integrating the above-mentioned features allowed the identification of patients with low-risk of disease-specific death with 100% sensitivity.
Conclusions: We demonstrate an automated machine learning workflow that captures the cellular interactions present in the tumour microenvironment and which, may lead to improved and personalised clinical decision making.

Intestinal Tumour Modelling and DNA Damage: Investigating the Interaction of Deficient DNA Mismatch Repair and Ethanol in Colorectal Carcinogenesis

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Lynch Syndrome (LS) confers inherited cancer predisposition due to germline mutations in one of the DNA mismatch repair (MMR) genes. MMR is a DNA-damage repair pathway involved in the removal of base mismatches and insertion/deletion loops caused by several endogenous and exogenous factors. Loss of MMR through somatic alteration of the wild-type allele in LS results in defective MMR (dMMR). Ethanol and its metabolite acetaldehyde are classified as group one carcinogens by the IARC. Aldehydes are very reactive molecules that constitute a serious threat to cellular integrity by causing a range of DNA lesions. However, DNA repair pathways responsible for correcting such lesions remains unknown. We hypothesized that MMR is involved in the repair of certain forms of ethanol/acetaldehyde-induced DNA damage. In this study, we aim to determine if there is a gene-environment interaction between dMMR and ethanol/acetaldehyde that accelerates colorectal tumourigenesis. We used a conditional Msh2 knockout mouse model that mimics the LS patients' pattern of MMR gene inactivation. The LS model mice (6-8 weeks of age) were fed either with 20% ethanol in drinking water or normal drinking water. Most of the ethanol-treated mice demonstrated large intestinal hyperproliferation, adenoma formation and, in some cases, invasive adenocarcinoma within 7 months (6/10), compared with one case of intestinal tumour formation after 16.5 months in the water-treated mice (1/10). The quantification of the dMMR crypts in LS mouse colon has shown an increased number of dMMR foci in ethanol-treated mice compared with the control group. Preliminary results indicate that long-term ethanol treatment induced acceleration of dMMR-driven large intestinal tumour formation. Possible mechanisms may include ethanol-induced mucosal crypt epithelial proliferation and ethanol/acetaldehyde mediated DNA damage that would usually be repaired, at least in part, by DNA MMR.
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Differences in Negative Lymph Node Size in Rectal Cancer Resections are Related to Patient's Immune Reaction

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Introduction: The number and size of lymph nodes without metastasis (LNneg) is an independent prognostic factor in rectal cancer (RC) patients. It is unclear what influences LN size in RC patients. To explore whether there is a relationship between LN size and LN architecture in RC patients we focussed our pilot study on LNnegs using haematoxylin/eosin (H&E) stained slides.
Method: 50 RC patients treated between 2012 and 2015 with surgery (n=17) or neoadjuvant treatment (NAT) followed by surgery (n=33) were included. All H&E slides were digitalised and LNnegs were outlined manually using image analysis software to calculate the LN area. In addition, LNs were reviewed under the microscope with a 2.5x objective to establish number of primary and secondary lymphoid follicles and presence of intranodal fat (inF). LNnegs were grouped in five groups based on primary and secondary follicle count. The relationship between LN morphology and LN area was analysed.
Results: 677 LNnegs were analysed. The median size of LNs with NAT is 98.48mm^2 (range 2.74mm^2 – 1107.71mm^2) compared to 119.33mm^2 (range 9.26mm^2 – 1214.89mm^2) in patients without. 150 (22.5%) LNnegs contained inF. Presence of inF was related to smaller LNneg area in patients after NAT (median (range) LNneg area without inF 103.9mm^2 (3.5mm^2 – 1007.7mm^2) versus 82.2mm^2 (2.7mm^2 – 642.9mm^2) with inF) p=0.04. Increasing numbers of lymphoid follicles were related to larger LNneg area in all patients (p < 0.05).
Conclusion: This pilot study in LNnegs from RC resections showed that patients with larger LNneg appear to have higher number of lymphoid follicles and interestingly less inF. This study seems to confirm a previously proposed interaction between the immune system and fat tissue. Thus, larger LNs in cancer patients could be related to patient prognosis and treatment response, this need to be investigated in future studies.

The Relationship Between Tumour Immune Profile and Response to FOLFOX-Based Pre-Operative Chemotherapy in the International Phase III FOxTROT Trial

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Recent strong evidence suggests that the immune system influences cancer prognosis in a number of cancers including colorectal cancer. The prognostic importance of tumour infiltrating lymphocytes (TILs) in colon cancer has long been recognised. However, the importance of TILs and other immune cells following preoperative chemotherapy is unknown and a validated methodology for assessment is lacking. Patients were randomly assigned in a 2:1 ratio to pre- and post-operative chemotherapy comprising three 2-week cycles of FOLFOX then surgery followed by nine more cycles, or to post-operative chemotherapy consisting of surgery followed by twelve 2-week cycles. H&E slides were collected for central pathological review in 904 out of 1052 cases (86%). The primary tumour immune profile was assessed for TILs (stromal and intratumoural), neutrophil and eosinophil infiltration at the invasive edge, tertiary lymphoid structures (TLS), abscess formation and necrosis. In addition, we assessed the area of all lymph nodes (LNs) identified and the area of metastatic tumour deposits in LNs. Significantly higher numbers of stromal TILs (14% vs. 9%, p<0.0001) and eosinophils (6% vs. 3%, p<0.0001) were observed in the pre-operative group, however, intratumoural TILs were equivalent (5% vs. 5%, p=0.63). Significantly lower numbers of neutrophils (5% vs. 10%, p<0.0001) and reduced abscess formation (11% vs. 21%, p<0.0001) was seen in the pre-operative group. The average area of all uninvolved LNs was smaller in the pre-operative group as was the average tumour area in metastatic LNs. There were no significant differences in TLS between the two groups.
This is the first study of the tumour immune profile in colon cancers treated with pre-operative chemotherapy, and demonstrates fascinating differences in immune cell populations between the chemotherapy and control groups. Ongoing work will assess the importance of tumour immune cell populations in the chemotherapy group according to response.

The Effect of Pre-Operative FOLFOX Chemotherapy in Advanced Colon Cancer on Histopathological Features: Analysis of the International Phase III FOxTROT Trial

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FOxTROT is the first international phase III randomised clinical trial to evaluate the effectiveness of pre-operative chemotherapy in locally advanced colon cancer. Patients were randomly assigned in a 2:1 ratio to pre- and post-operative chemotherapy or to post-operative chemotherapy alone. Central pathological review of the core histopathological features was performed in 904 out of 1052 cases (86%). Additional histopathological features were also assessed during the central review including restaging according to TNM8, extra-nodal spread, tumour budding, poorly differentiated clusters (PDC), and tumour deposits (TDs). All histopathological features generated during the central pathological review were then compared between the pre-operative chemotherapy group and control arm. Pre-operative chemotherapy was associated with a significant reduction in tumour diameter (40mm vs. 51mm, p<0.0001), lower pT stage (pT0-pT2 rate 18% vs 8%, p<0.0001), lower pN stage (pN0 rate 64% vs. 52%, p=0.0002) and greater R0 rate (99% vs. 96%, p=0.02). Apical node metastases were less common in the pre-operative chemotherapy group (3% vs. 8%, p=0.002) as was extracapsular nodal spread (8% vs. 19%, p<0.0001), intramural venous invasion (20% vs. 33%, p<0.0001), extramural venous invasion (35% vs. 44%, p=0.004) and lymphatic invasion (46% vs. 55%, p=0.002). There was no significant difference in perineural invasion (12% vs. 14%, p=0.69). The percentage of the patients with high grade budding was lower in preoperative group (5% vs. 14%, p<0.0001), however, no significant difference was found in the number of PDCs (4.0 vs. 4.7, p=0.08). Pre-operative chemotherapy in locally advanced colon cancer is associated with significant effects on the primary tumour leading to improved complete resection rates. There is also a significant reduction in many high risk features and mechanisms of metastatic spread, all of which are expected to lead to an improvement in patient outcomes.

An Assessment of the Mutation Rate or Normal Colorectal Epithelium in Patients with Cancer Compared to Patients Without

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It is thought that half of somatic mutations present in colorectal cancers have arisen previously in the epithelium. In order to become fixed, these mutations must occur in colonic stem cells which can then replace the crypt. To study the mutation rate we used a neutral clonal marker, MAO-A. It is located on the X chromosome and truncating mutations result in loss of staining of the protein with immunohistochemistry allowing for direct visualisation of fixed mutations. Normal colonic mucosa was examined from cancer patients (cancer-associated normal, CAN) N=9 and patients who had resections for non-neoplastic and non-inflammatory indications (non-neoplastic normal, NNN) N=6. Slides were stained for MAO-A and digitally scanned. The total mucosal area was measured and 300 random points were scored as either epithelium, lamina propria or non-relevant. Next 50 randomly selected crypts per slide were measured to estimate the average crypt size and the total crypt number. For the CAN group the average mutation rate was 1 in 2646 crypts. The 6 samples of NNN had an average mutation rate of 1 in 6737; this meant a 2.6 fold difference for CAN compared to NNN. This difference was significant (p=0.0198). The average age of the patients in the two groups was no significantly different; CAN=72years, NNN=69years, p=0.516. The mutation rate increased with increasing age in both groups; the lowest mutation rate was 1 in 12842 for a patient aged 44 and the highest rate was 1 in 901 crypts for patient aged 88. Mutations accumulate throughout the colorectal epithelium during a person's lifetime and are present in histologically normal mucosa before cancer occurs. By using a neutral clonal marker, MAO-A, we have shown a difference in the mutation rate of the normal mucosa from patients with cancer and without. Although a relatively low sample size, we have still demonstrated a clear difference of 3.6-fold. This may indicate more genetic damage occurring in the colorectum of cancer. patient

Lynch Syndrome Screening for Colorectal Cancer: The Provisional Results of a Two-Year Regional Programme

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Around 3% of colorectal cancer (CRC) is associated with Lynch syndrome (LS). All newly diagnosed CRC should be screened for LS according to NICE guidance yet most centres do not routinely offer this service due to lack of identified funding. Here we report the two year results of a regional LS screening programme offered to 16 multidisciplinary teams serving a population of 5.7 million. Screening was available for all patients diagnosed with CRC over the age of 50 years. Local centres were asked to refer a formalin fixed paraffin embedded tumour block (preferably biopsy) to the central laboratory for MLH1, PMS2, MSH2, and MSH6 immunohistochemistry. This was followed by BRAF codon 600 pyrosequencing and MLH1 promoter methylation analysis as appropriate. Twelve hospitals referred blocks from 2415 patients between May 2017 and March 2019 (66% biopsies/polyps and 34% resections). In total, 335 showed deficient mismatch repair (13.9%) of which 253 showed loss of MLH1/PMS2 (10.5%), 20 loss of MSH2/MSH6 (0.83%), 15 loss of isolated PMS2 (0.62%) and 13 loss of isolated MSH6 (0.54%). In addition, less common combinations of protein loss were observed in 34 cases (1.4%). Of the cases showing MLH1 loss, 75.5% contained a BRAF mutation. Of the wild type BRAF patients, 82.3% showed MLH1 promoter hypermethylation. Following screening, 68 patients were strongly recommended for LS germline testing (2.8%). Through a large regional LS screening programme, we have demonstrated a 14% rate of deficient mismatch repair and recommended 3% of patients to be referred for genetic counselling and consideration of germline testing. This will ensure that patients and their families are managed appropriately with regard to adjuvant chemotherapy decisions and the identification and subsequent management of LS.

Analysis of TP53 Mutation Status and p53 Protein Expression in a Subset of 296 Patients Enrolled in the FOCUS4 Clinical Trial

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It is well recognised that the correlation between *TP53* mutation status and protein expression is limited. With *TP53* mutation status being determined by next generation sequencing (NGS), to guide patient randomisation in the FOCUS4 mCRC clinical trial, we took the opportunity to also assess, in parallel, p53 protein expression. *TP53* mutation status was determined using the Qiagen Clinically Relevant GenePanel, and run on a MiSeq sequencing platform. Protein expression was determined using the DAKO mouse monoclonal anti-human p53, clone DO-7. Both mutation and protein expression data were available for 296 patients. 212/296 (71.6%) of tumours contained a *TP53* mutation, with the remaining 84/296 (28.4%) being wild-type (WT). The ratio of biopsy to resection tumours in the *TP53*-WT cohort was 65.5:34.5, and in the *TP53*-mutant cohort was 52.8:47.2 (p=0.047). 60.8% of mutations were missense, 19.3% nonsense, and 11.3% in-frame deletions, with in-frame variants, intronic substitutions and splice acceptor variants constituting 2.8%. 5.7% were unknown. Minimum and maximum mutant allele frequencies (MAFs) were 6% & 86% and 6% & 85% in biopsy and resection tumours respectively. 136/212 (64.2%) of *TP53*-mutant tumours showed protein overexpression, 48/212 (22.6%) were negative, 19/212 (9%) showed patchy staining, with the remainder showing cytoplasmic expression. The nonsense mutation c.916C>T p.Arg306Ter was present in 8/9 tumours displaying cytoplasmic staining. Patchy staining was seen in 42/84 (50%) of WT tumours, but 31/84 (36.9%) showed overexpression, 9/84 (10.7%) showed no expression and 2/84 (2.4%) showed cytoplasmic staining. We have demonstrated aberrant staining patterns in 91% of *TP53*-mutant tumours, however, we have also seen this in 50% of the *TP53*-WT tumours, clearly demonstrating that protein expression is not a suitable surrogate when determining mutation status.

Investigating the Effects of Radiotherapy on the Bowel Cancer Microbiome: Reanalysing the MRC CR07 Trial

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Purpose of study: In recent years, the importance of the gut microbiome in the biology of colorectal cancer is being increasingly recognised. However, there is a paucity of data with which to build a baseline measure of what constitutes a cancerous microbiome. Fortunately, bacterial sequences can be detected in existing genomic data, such as that produced for the MRC CR07 trial, a phase III trial studying total mesorectal excision +/– short course pre-operative radiotherapy.
Methods: Low coverage whole genome sequencing data of FFPE resection samples from 470 CR07 patients (224 RT+ versus 246 RT–) was reanalysed using the PathSeq algorithm, which searches for bacterial sequences.
Summary of results: A median of 9,783,000 sequence reads were processed per sample of which median 9139 were assigned to bacterial taxa. The numbers of bacterial reads did not significantly differ between the RT+ and RT– arms. Common gut bacteria such as *Bacteriodes* and taxa associated with cancer such as *Fusobacterium* were frequently observed. The frequencies of the taxa varied between individuals, but the frequencies within the cohort as a whole were similar to published results. Comparing the RT+ versus RT– arms, 19 taxa were differentially abundant. These were mostly isolated species, but did include several taxa in the *Firmicutes* phylum, found in the treated arm. Some of these, such as *Peptiniphilus* are known to infect wounds and other inflamed tissue.
Conclusions: We have shown that old data can be re-analysed in order to study bacterial populations. Any next-generation sequencing data could be examined in this way, or any trial cohorts could be sequenced with this analysis plan. In the case of the CR07 trial, we have shown that the profiles obtained previous findings, with a number of taxa linked to bowel cancer. The two arms of the trial were distinguished by taxa known to inhabit damaged tissue, which is entirely plausible given the radiotherapy treatment regime.

The Potential Role of Raman Spectroscopy in Predicting Response to Pre-Operative Radiotherapy in Rectal Cancer

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High risk rectal cancer patients frequently receive neo-adjuvant radiotherapy (RT). However, there are currently no approved methods of predicting patient response, and a significant proportion of patients show no response at all. Raman spectroscopy is a non-destructive technique able to provide the unique chemical fingerprint of tissues, detecting changes in molecular composition prior to visible morphological changes. Using Raman spectroscopy, we aimed to build a classification model capable of predicting patient response to pre-operative short course RT, in order to guide personalised patient treatment. High quality Raman spectra were collected from formalin fixed paraffin embedded (FFPE) biopsy and resection samples from 10 rectal cancer patients, who had received pre-operative short-course RT. Cases were selected based on response to RT, determined by calculating the percentage reduction in tumour cell density (TCD), following pre-operative RT. Regions of interest were selected using digitalised H&E stained sections and the spectra collected from the corresponding unstained sections using an inVia Raman confocal inverted microscope. The same regions were then re-mapped using a Renishaw benchtop RA816 Raman spectrometer. The cases that showed a poor response to pre-operative RT had a TCD reduction of less than 36%, and those showing a good response had a reduction in TCD greater than 75%. Our preliminary results, as presented in 2018 showed a principal component analysis - linear discriminant analysis (PCA-LDA) classification model was able to differentiate areas of tumour and stroma within patient resections. These results have since been supported by data from additional patient samples, and current analysis is underway to confirm reproducibility of the model using an alternative Raman spectrometer. From this, further work is ongoing to build a classification model capable of predicting response to pre-operative RT, from spectra collected from patient biopsies.

Central Pathological Review of the International Phase III FOxTROT Trial: Comparison with the Local Pathological Evaluation

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FOxTROT is the first international phase III randomised clinical trial to evaluate the effectiveness of pre-operative chemotherapy in locally advanced colon cancer. Patients were randomly assigned in a 2:1 ratio to pre- and post-operative chemotherapy or to post-operative chemotherapy alone. Local pathology evaluation was undertaken in 94 centres in the UK, Sweden and Denmark. H&E stained slides were available for central pathological review in 904 out of 1052 cases (86%). All slides were scanned at 20x magnification using an Aperio XT and viewed digitally using Aperio ImageScope. The central assessment was performed blinded to the original local evaluation by a single observer for all of the microscopic pathological features captured locally on the case report form, where these were assessable. Staging was performed according to TNM version 5. The overall concordance rates between the central pathological review and the local pathological evaluation were very good. Specific items assessed and the associated concordance included: pT stage (95%), pN stage (94%), resection margin status (99%), intramural venous invasion status (89%), extramural venous invasion status (89%) and Dukes' stage (96%). The local pathological evaluation in the FOxTROT trial has been confirmed to be carried out to an excellent standard across a large number of centres in three countries. Concordance with the central pathological review is excellent for most histopathological features, with a slightly lower rate of agreement for venous invasion, which is recognised to be frequently missed by pathologists. Central pathological review should ideally be built into all clinical trials using histopathological endpoints to confirm the quality of local reporting.

Alcian Blue: A Rediscovered Biomarker of Poor Prognosis in Gastric Cancer Patients

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Background: Gastric carcinoma (GC) is one of the major causes of cancer related deaths worldwide. GC mucin phenotype has been related to tumour invasion and genetic alterations. However, the relationship between mucin phenotype, clinicopathological variables and GC patient survival remains a matter of debate.
Methods: Tissue microarrays from 709 GC resections were stained immunohistochemically for MUC2 (intestinal-type mucin) and MUC5AC (gastric-type mucin) and histochemically for Alcian Blue (AB) periodic acid-Schiff (PAS) (acidic and neutral mucin). Stainings were scored using a 10% cut off to define positivity. The relationship between marker, clinicopathological variables and survival was analysed.
Results: 16% GC were MUC2 positive, 36% MUC5AC positive, 6% AB positive, 3% PAS positive and 11% AB and PAS positive. 4% GC were triple positive, 49% triple negative. Expression of MUC2, MUC5AC and ABPAS staining was related to GC histological phenotype ($p<0.05$). AB positivity was related to deeper invasion ($p=0.006$) and poorer grade of differentiation ($p<0.001$). Patients with AB negative GC (n=543) survived significantly longer than those with AB positive GC (n=112), $p=0.001$. Survival of patients with PAS positive GC (n=20) was similar to those with AB negative GC. There was no relationship between MUC2 or MUC5AC and patient survival.
Conclusions: This is the first study to show that patients with AB positive GC are more likely to have locally very advanced disease, poorly differentiated GC and poorer survival. The underlying biological mechanisms related to the switch from PAS positive mucin in the normal gastric mucosa to acidic/intestinal type AB positive mucin in GC are currently unclear and warrant further investigations.

Routine CT Scan One Year After Surgery can be Used to Estimate the Level of Arterial Ligation in Colon Cancer Surgery and May be More Accurate Than Standard Histopathological Measurements

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Complete mesocolic excision (CME) is increasingly being used to optimise colon cancer surgery and involves central ligation of the tumour feeding vessel at its origin. We have previously shown that measuring the arterial stump on a CT-scan performed two days after surgery renders a reliable estimate of the level of artery division. The aim of this study was to identify and measure the arterial stump on a routine CT-scan one year after surgery, and compare the results with those obtained two days after surgery.
52 patients had surgery for colon cancer followed by a CT-scan two days after surgery. One year after surgery, 47 patients had a CT-scan as a part of the standard follow up. Both the images were evaluated by the same specialist radiologist. The vessels were identified and measured from the origin to the point of ligation. In 38 cases (81%) we were able to identify and measure the arterial stump on both scans. Overall, we found no difference in the length of the vessel one year after surgery compared to the length after two days (mean difference -1.7 mm; 95% CI (-3.8 to 0.5 mm), p=0.13). However, vessels categorized as thrombosed or as a fibrotic line were shorter after one year (mean difference -4.5 mm; 95% CI (-8.9 to -0.1 mm), p=0.05). Routine CT-scans obtained one year after surgery can be used to estimate the level of arterial division following colon cancer surgery. These measurements are highly likely to give a more accurate impression of the level of arterial division when compared to standard histopathological measurements performed on the specimen, given that vascular anatomy is highly variable and that histopathologists cannot comment on what has been left behind in the patient. The level of arterial division (assessed radiologically) should be combined with the plane of mesocolic surgery (assessed pathologically) to ultimately determine the quality of the specimen.

The Creation and Validation of a Global Microbiome Colorectal Cancer Research Network

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Research investigating the colorectal cancer (CRC)-associated microbiome has been almost entirely conducted in 'Western' high CRC incidence countries. Funded by the UK Academy of Medical Sciences Global Challenge, we established a global research network to compare the CRC-associated microbiome of high (UK and Argentina), intermediate (Chile) and low (India and Vietnam) CRC incidence countries. Faecal samples were collected using bowel cancer screening cards from 10 CRC patients and 10 healthy volunteers from each country and transported to the UK at room temperature. Replicate control samples from 5 UK healthy volunteers were generated to assess for the effect of transportation and storage abroad. V4 16SrRNA sequencing was performed. Here we present the results of samples from India, Vietnam and Argentina, as samples from Chile were not available at the time of sequencing, but are currently being processed. There were no significant differences in bacterial community between any of the UK replicate controls, indicating that transport and local storage of samples does not alter microbiome results. There was no significant difference in the alpha diversity of samples from the different countries, but the beta diversity differed significantly for both weighted and unweighted UniFrac distances. Beta diversity differed significantly between the combined healthy volunteer group and the combined CRC group for unweighted but not weighted UniFrac distance, indicating that rare taxa account for this difference. These include *Fusobacterium*, *Parvimonas*, *Porphyromonas* and *Escherichia-Shigella* which are CRC-associated bacteria described in the literature. We have demonstrated a robust method of conducting global CRC microbiome research. The microbiome differs by country and differences in rare taxa exist between the combined microbiome of healthy volunteers from India, Vietnam and Argentina compared with CRC patients. Plans are underway to expand the network and sample collection.

The Use of Stochastic Optical Reconstruction Microscopy (STORM) in Formalin Fixed Paraffin Embedded Tissue

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Background: Colorectal cancer (CRC) with amplification/over-expression of cell surface receptors or ligands are specifically targetable (e.g. HER2 and anti HER2 therapy, EGFr and anti EGFr therapy) but most patients selected for these therapies fail to respond due to unknown mechanisms of resistance. Developments in advanced fluorescence microscopy have made it possible to resolve protein localisation at up to 5nm resolution. In this pilot study we aimed to develop a robust routine methodology for Formalin Fixed Paraffin Embedded tissue (FFPE) that exploits this technology and explore its potential for visualisation of ligand-receptor pathways in CRC to increase our understanding of resistance mechanisms at a cellular level.
Methods: To establish the protocol on FFPE tissue we selected colorectal cancer cases with strong HER2 and negative HER2 receptor expression. Previously described cell culture protocols (Creech et al. 2017) were modified, optimised and imaged by confocal microscopy using HER2 (1:250) with affinity purified secondary antibody (1:500). The optimised protocol was used in 3D dSTORM on FFPE.
Results: Protocols for HER2, EGFR, RAB5 and RAB11 expression on FFPE samples were determined using the confocal microscope. In 3D dSTORM, high levels of HER2 were localised to aggregates in the membrane and lower levels in the cytoplasm. Further work will focus on imaging and quantification of further components of the MAPK/ERK pathway in 3D dSTORM and imaging multiple proteins in combination to assess ligand-receptor and receptor-adapter interactions as well as receptor cycling. STORM microscopy opens up subcellular microscopy in FFPE to Histopathologists.

Comparison of Two Methods to Analyse Components of the Microbiome from FFPE CRC Tissue: Low Coverage WGS and qPCR

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Microbiome research is rapidly advancing, with particular focus on the association with colorectal cancer (CRC). Prospective studies, which typically perform metagenomic or 16SrRNA sequencing on faeces or fresh-frozen tissue from CRC patients and healthy volunteers, have identified specific bacteria as being significantly enriched in CRC patients. One, *Fusobacterium nucleatum* (*F.nucleatum*), is associated with chemo-resistance and poor prognosis. Additional clinically-relevant associations could be discovered through the assessment of archival FFPE CRC clinical trial material. For trials which have had low coverage whole genome sequencing (LCWGS) performed, it is possible to interrogate the WGS data for reads which map to the genomes of the specific bacteria of interest or to perform quantitative polymerase chain reaction (qPCR). Here we compare the two methods, taking TaqMan qPCR as the gold standard.
qPCR amplification of *F.nucleatum* was performed in triplicate using DNA from 148 CRC samples, for which LCWGS (less than 1x) data was also available. qPCR amplification of the human prostaglandin transporter gene was used as a reference and (2^-deltaCt) was calculated. The LCWGS data was aligned to a composite artificial genome of human and bacterial sequences using BWA aligner and *F.nucleatum* load was calculated.
The Pearson correlation coefficient for the relative abundance of *F.nucleatum* between the two methods was strong (0.74). Compared with qPCR, LCWGS had a sensitivity of 66% (63/96) and positive predictive value of 69% (63/91) for the detection of *F.nucleatum*. We have shown that existing LCWGS data from FFPE CRC clinical trial material is valuable for the investigation of the presence and relative abundance of *F.nucleatum* but has a lower sensitivity than TaqMan qPCR. We recommend performing analysis of existing LCWGS data from archival CRC clinical trial material in order to generate hypotheses, with subsequent validation by TaqMan qPCR.

When To Biopsy? Compliance With Local Guidance for Effective Endoscopic Biopsy Practice

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Purpose of study: Our local guidance 'When to Biopsy' was first written in 2002 following the Royal College of Pathologists 'Specimens of limited clinical value', to ensure appropriate use of endoscopic biopsy resources. It has been updated periodically in discussion with endoscopists, and is displayed in all local and external endoscopy suites. New BSG/AUGIS standards in upper gastrointestinal endoscopy were published in 2017 (Beg S et al, Gut 2017). Before updating our own biopsy guidance, we audited compliance with existing guidance.
Method: The endoscopic biopsies reported during 1 week in March 2018 were assessed prospectively for compliance with the guidance provided. The indication, site, number of biopsies, endoscopist and pathologist were recorded. Following this, further consecutive biopsies taken in the independent endoscopy units were assessed in the same way, to compare with the Trust cohort.
Summary of results: 79% (89/112) of Trust endoscopy suite biopsies conformed to the guidance. Of the 23 that did not, 16 were an incorrect or incomplete series, and 7 had no clinical indication. 41% (29/70) of external endoscopy unit biopsies conformed, significantly less than within the Trust ($p<0.0001$). Of the 41 that did not, 11 were an incorrect or incomplete series, and 30 had no clinical indication. An incomplete series for diagnosis of microscopic colitis was the most common 'incorrect/incomplete series'.
Conclusions: Our audit showed a 79% compliance within the Trust. Overall, improved compliance would not have affected biopsy numbers. Compliance was lower in endoscopy outsourced to independent units, usually due to biopsies of normal mucosa without clinical indication. We have updated our local guidance in line with BSG/AUGIS standards and plan to re-audit practice. There is a national shortage of pathologists and focus on efficient working – we have found our guidance effective, but needs to be provided in the context of close working with endoscopists.

Stage is Not Predictive of Time to Recurrence in Colorectal Cancer

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Introduction: Colorectal cancer (CRC) has a variable presentation, treatment options and prognosis depending on multiple underlying factors such as site, molecular signature and invasion. While many of these risk factors have been identified and investigated, their interactions and effects on prognosis are not fully understood. The recent update to Royal College dataset incorporated an updated understanding of a number of these risk factors; we sought to test a number of these risk factors and others using a large tissue-linked pathology database.
Methods: We identified 1000 recent cases of colorectal cancer from our local pathology database, including full pathology reports of surgical biopsies and resections (initial diagnosis from 2008 to 2014). Patient demographics and follow-up data were gathered from the hospital patient information system. Survival analyses including overall survival, cancer-free survival and cancer-related mortality were performed, according to known risk-factors for validation. We tested the effect of overall stage on time to recurrence, overall survival and cancer-related mortality.
Results: High overall stage was highly predictive of adverse outcome, with people with stage 1 CRC having a mean survival of 101 months (95% CI: 95 – 108 months), stage 2: 85 months (95% CI: 80 – 89), stage 3: 75 months (95% CI: 70 – 80) and stage 4: 34 months (95% CI: 28 – 40). Similarly, cancer related death was strongly predicted by higher overall stage. While higher stage predicted number of recurrences, we found that time to recurrence was not related to overall stage. The mean time to recurrence for all stages was 22 months (95% CI: 20 – 24) and after 36 months the likelihood of recurrence was less than 10%.
Conclusion: Almost all colorectal cancer recurrences occur within 3 years of primary diagnosis. Although stage is associated with higher risk of recurrence, it does not predict time to recurrence.

Presence of Vascular Invasion in Colorectal Cancer Correlates with TNM8 Nodal Status Classification

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Purpose of the study: Vascular invasion (VI) is an important prognostic marker in several malignancies including colorectal cancer (CRC). The correlation between the VI status and the revised nodal stage of CRC as per TNM8, was investigated in this study.
Methods: 1000 consecutive cases of CRC from a tertiary centre, diagnosed between 2008-2014 were investigated for VI profiles from histopathological reports on IT records and results correlated to clinicopathological variables including the revised nodal stage as defined by the TNM8 classification.
Summary of results: 483 of 985 (49%) cases showed extramural vascular invasion (EMVI), the incidence of which within stages 1-4 of CRC was 1%, 44%, 68%, 72% respectively ($p<0.001$). Positive VI status was associated with higher grade, nodal status, distant metastases and recurrence ($p<0.001$). Within the revised nodal stages as per TNM8, positive VI status was seen in 34% (N0), 58% (N1a), 64% (N1b), 85% (N2a), 96% (N2b) of cases ($p<0.001$) Intramural VI (282/916) also correlated with tumour stage ($p<0.0001$), distant metastases ($p=0.003$) and recurrence ($p=0.002$). Within the revised nodal stages as per TNM8, intramural VI was seen in 25% (N0), 33% (N1a), 30% (N1b), 46% (N2a), and 65% (N2b) cases ($p<0.001$). Presence of intramural VI in the absence of EMVI however did not correlate with lymph node metastases and recurrence. EMVI conferred poorer 5 and 10 year overall and cancer specific survival ($p<0.001$) and in N0 cases, was correlated with worse overall survival ($p=0.001$).
Conclusions: Overall, this study shows that both intramural and extramural VI in CRC have strong correlations with the increasing nodal stage, as defined by the TNM8 classification. Revalidation of prognostic associations of VI with clinicopathological variables and survival in novel datasets of CRC establishes confidence in the the cohort for subsequent biomarker/molecular analysis.
Supported by the Nottingham Molecular Pathology Node.

A Novel 3D Cell Culture System Demonstrates Induced Pluripotent Stem Cells Driven to Intestinal Differentiation are Capable of Spontaneous Crypt Assembly

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Introduction: Induced pluripotent stem cells (IPSC) are an important tool for studying development and disease and give the potential for personalised tissue regeneration in future. While more is known about the fundamental signalling pathways driving IPSC to differentiate along specific tissue lines, much of the evidence for differentiation is derived from molecular assays or organoid assays. Although these form vital evidence, molecular assays lack the morphological context of cells in a 3D environment and organoids do not always faithfully recapitulate the tissue organisation of cells within a luminal organ. The aim of this study was to develop a long-term 3D culture system of the intestinal mucosa and submucosa from IPSC, which would allow conventional histological and immunohistochemical methods to be used for analysis.
Methods: IPSC were driven to intestinal differentiation using a novel serum-free protocol incorporating Wnt and Nodal pathway activators for 8 days. These intestinal progenitors were seeded onto the surface of pre-cast 3D culture gels consisting of purified extracellular matrix components. Cells were maintained in culture medium supplemented with growth factors promoting growth and maturation. After 4 weeks of culture, the gels were formalin-fixed and paraffin-embedded and sectioned for histology and immunohistochemistry for intestinal markers.
Results: Cells formed a polarised epithelium on the surface of the gel with an underlying mesenchymal component. The mucosa was capable of spontaneous intestinal crypt assembly. Cells showed expression of a range of intestinal markers in the epithelium and mesenchymal markers in the submucosa. There was no evidence of aberrant differentiation or retained pluripotency.
Conclusion: Using a novel 3D culture, it has been possible to demonstrate that IPSC driven to intestinal differentiation are capable of forming mature intestinal mucosal and submucosal structures.

Faux-Multiplex Immunohistochemistry (fm-IHC) to Delineate Biomarker Territories and Biomarker Co-Localisation in Colorectal Cancer

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Colorectal cancers (CRC) are heterogeneous. Mapping the geographical expression pattern and the co-localisation of biomarkers within a tumour would allow activated pathways and cellular communities to be defined. This is possible with immunofluorescence but multiplex immunohistochemistry (IHC) is beset with problems such as antibody cross reactivity and tissue degradation. In this project, we aim to build on the registration facility of Histogenic Molecular Mapping (HMM) to establish faux-multiplex immunohistochemistry (fm-IHC) as a novel approach for multiple biomarker analysis. Sequential whole tissue sections (WTS) of FFPE blocks of CRC were subjected to IHC for stromal, epithelial, and various immune cells markers. The staining was carried out automated slide stainer (Ventana) and digital slides were produced using Aperio slide scanner (Leica). For each block, all consecutive IHC images were registered to the middle one in the block to establish accurate correspondence across all biomarkers (a particular landmark in one registered IHC image can be found at the same position in all the other registered image). Each registered image was then downsampled by a factor of 10 to account for small differences across consecutive images and small registration inaccuracies. Subsequently, the images were automatically colour separated and thresholded to obtain expression maps and to establish correlations across combination of biomarkers. Fm-IHC analysis of BerEP4, CD3 and CD20 was undertaken in 20 CRC which were mismatch repair deficient (dMMR, n=10) or proficient (pMMR, n=10). The intra-epithelial lymphocytes (BerEP4+/CD3+ frames) and the stromal lymphocytes (BerEp4-/CD3+ frames) could be identified. Overall, dMMR tumours had a higher density of CD3+ cells but there was no difference in the compartmental distribution (intra-epithelial vs stromal). There was significant negative association of CD3 and CD20 in the medulla and germinal centre mantle of lymph node.

Hepatocyte Nuclear Factor 4A is a Novel Tumour Suppressor in Pancreatic Cancer

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Abstract withdrawn at the request of the authors

Cten Ability to Induce Migration is Dependent on its SH2 Domain

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Cten (Tns4) is a member of the tensin family and acts as an oncogene in colorectal cancer where it enhances EMT, migration and invasion but not proliferation. It is normally localized in the cytoplasm but it was found that it translocates to the nucleus where it binds to beta-catenin. The mechanism by which Cten induces EMT, invasion and migration is not fully understood. The structure of Cten has an SH2 domain. The SH2 domain is found in different proteins and has a very important role in regulating signaling cascades. SH2 domains play a vital role in interacting with different receptors in several cancers. Upon interaction, signals are transmitted through several pathways to induce growth and metastasis in different types of cancers. We performed Site-directed mutagenesis to delete the SH2 domain of Cten plasmid. We then transfected HCT116 with Empty vector, wild-type Cten, and Cten(Δ SH2). This was followed by functional assays, protein extraction and western blot. Our data from functional assays confirms Cten overexpression is inducing cellular migration but not proliferation in HCT116. Moreover, we have discovered that the SH2 domain is essential for Cten to induce migration. Moreover, our data illustrate that Cten regulates E-cad, Snail, N-cad and ROCK1 through SH2 domain. We have shown by western blot that Cten is Upregulating Snail, ROCK1 and Ncad and downregulating Ecad. When the SH2 domain was deleted, the expression of these proteins was restored.
Conclusion: Cten induces migration in Colorectal Cancer through its SH2 domain.
Cten regulation of downstream targets is controlled by its SH2 domain

Evolution of Chronic Lymphocytic Leukaemia / Small Lymphocytic Lymphoma

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Introduction: Chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL) is common B cell malignancy with heterogenous behaviour. Certain cases present with atypical morphological/immunophenotypic features; others develop these features over time. Many such features indicate poorer prognoses. The current study aims to characterise these CLL variants.
Methods: Retrospective review of CLL cases performed in Hammersmith Hospital archives. Atypical CLL (atCLL; n=58; cleaved nuclei), aggressive CLL (agCLL; n=17; diffuse/confluent proliferation centres) and Richter transformation to diffuse large B cell lymphoma (RT; n=14) cases were compiled. Comparisons made with classic CLL (cCLL; N=71). Available data collected on the immunophenotype on paraffin-embedded tissue and cytogenetics.
Results: Mean age at diagnosis was 71.1 years. No significant age difference noted across CLL types. Tissue from lymph node (48%, N=78), bone marrow (40%, N=64) and other sites (11.3%, N=18) with more extra-nodal/extra-marrow sites in RT (Chi Square, $p<0.001$). A significant difference in Ki67 index noted across the groups (ANOVA, F=66.57), $p<0.001$). Post hoc comparisons showed RT Ki67 (M=70, SD = 16.4) was higher than agCLL (M=54.3, SD=13.2) which was greater than either atCLL (M=22, SD=9.7) or cCLL (M=22.9, SD=10.1). There was a significant difference in CD5 (Chi-Square, $p<0.001$; lower in RT), CD23 (Chi Square, $p<0.001$; lower in RT), MUM1 (Chi Square, $p<0.01$; higher in RT and agCLL) and BCL6 (Chi-Square, $P<0.001$; higher in RT and agCLL) expression across the groups. No significant difference in Lef-1, CD38, BCL3, EBER, Pax5, CD21, IgM or IgD expression was seen across the groups. No significant differences in trisomy12, del(11q), del(17p) or del(13q) between atCLL and cCLL.
Conclusions: Morphological CLL variants have corresponding immunophenotypic changes. Clinical correlation is underway. Further genetic testing will be necessary to define the precise genetic chages across these types.

OCT2 as a Diagnostic Immunohistochemical Marker in Rosai-Dorfman Disease

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Here we characterise a consecutive series of RDD cases diagnosed in our regional haematopathology diagnostic service, demonstrating that histiocytes in all RDD cases in our population are characterised by the strong expression of the transcription factor OCT2. A consecutive series of RDD cases diagnosed between 2008-2018 according to standard practice at the regional HMDS diagnostic service were identified. Diagnostic criteria were based on characteristic morphology and S100+ reactivity of sinus histiocytes. Staining for S100, CD163 , CD1a , CD68, Langherin and OCT2 with mouse mAb. Antigen retrieval on FFPE sections was carried out using DAKO PT links with high antigen retrieval buffer with antigen retrieval at 95°C for 15 min. DAKO Envision Flex+ kit was used with standard detection methods with DAB chromogen and haematoxylin counter stain. The average patient age at diagnosis was 43 years and six months (range 3 years to 81 years). There were slightly more female than male cases (62% female, n=16). 35% (n=9) cases had recurrent disease. 50% (n=13) cases had nodal disease at presentation, 38% (n=10) had extra-nodal disease and 19% (n=5) had both nodal and extra-nodal disease at presentation. The most commonly affected sites were head and neck 62% (n=16), breast 19% (n=5) and skin and soft tissue 31% (n=8). Of the cases stained for S100, 98% were positive, 76% were CD68 positive, 100% were positive for CD163. None of the cases stained for Langherin or CD1a were positive. Two patients with recurrent disease had IHC performed on their recurrence specimens, which also demonstrated clear nuclear positivity for OCT2. Control cases did not show strong nuclear positivity for OCT2. Here, we have demonstrated that OCT2 is a reliable immunohistochemical test for the diagnosis of RDD. This antibody is widely used in clinical practice so can be readily applied in this alternate diagnostic setting.

Identification of Suitable T-Cell Associated Transcripts for the Development of a New Veterinary Diagnostic Test for T-Cell Lymphoma

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Lymphoma is one of the most frequently encountered malignancies in veterinary practice, particularly in cats and dogs, but veterinary pathologists struggle with current diagnostic techniques to distinguish T-cell lymphomas from infiltrates of benign T-cells. Clonality studies are possible for cat and dog, as in human clinical pathology, but these are complex and time-consuming with variable success rates. In human pathology, we have developed a new chromogenic in situ hybridisation (CISH)-based assay for formalin fixed paraffin embedded histological samples to look for T-cell monotypia, in a manner analogous to kappa/ lambda for B-cells. We determine the ratio of the TRBC1: TRBC2 constant segments in T-cell populations. Significant skewing away from the normal 1:1 ratio indicates likely lymphoma. In order to apply this approach to veterinary samples, we set out to identify the animal sequences, to determine their relative levels of expression and to provide preliminary CISH-staining data. By aligning publicly available sequence data with the human TRBC1/2 sequences, the T-cell receptor constant regions, TRBC1 and TRBC2, were predicted for cat, dog and mouse. These were amplified from cDNA samples by PCR and confirmed by Sanger sequencing. Sequences from multiple animals showed greater polymorphism in cat and dog, with implications for CISH probe design. As for the human sequences, the 3' untranslated region shows the greatest variation between TRBC1 and TRBC2, making this a promising site for segment-specific CISH probe design. Q-PCR indicated a TRBC1: TRBC2 ratio close to 1:1 in cat, dog and mouse. TRBC1 and TRBC2 specific probes, produced by PCR and labelled with digoxygenin, gave excellent CISH staining for both TRBC1 and TRBC2 in lymphoid cells and on FFPE mouse spleen tissue. In summary, we have demonstrated that CISH-based detection of TRBC1/2 could have utility as a test for animal T-cell lymphoma as it is likely to have for human T-cell lymphoma.

MYC Translocation-Positive Diffuse Large B-Cell Lymphoma: The Clinicopathological Impact of Copy Number Gain of the Translocated *MYC* Allele

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MYC rearrangement occurs in ~10% of diffuse large B-cell lymphoma (DLBCL). This can occur as an isolated single-hit (SH) or, frequently, in association with *BCL2* and/or *BCL6* rearrangement known as double-hit (DH) or triple-hit (TH). DH and TH are associated with a poor prognosis and routinely screened for by fluorescence in situ hybridisation (FISH) at histological diagnosis. Often, FISH also demonstrates copy number change at the *MYC* locus including gain of extra copies of the translocated allele or amplification of the intact allele. The clinicopathological impact of these changes is unclear. We identified 21 cases of DLBCL with increased *MYC* copy number, including gain of 1-3 extra copies of the translocated *MYC* allele (*MYC/BCL2/BCL6*-TH=5, *MYC/BCL2*-DH=6, *MYC/BCL6*-DH=2, *MYC*-SH=4), or amplification (>4 copies) of the intact *MYC* allele without *MYC* translocation (MYC-amp). The median age was 65 years (range 43 - 83 years) and 57% were male. 6/21 cases showed blastoid morphology with a "starry sky" appearance on histological review. Immunohistochemistry revealed high MYC protein expression indices (≥60%) in the majority of cases (16/21) and GC-phenotype in 95% according to Hans algorithm. Targeted sequencing of a panel of 70 genes associated with DLBCL demonstrated frequent mutations in *KMT2D* (57% of cases), *TP53* (52%), *FOXO1* (33%) and *MYC* (33%). Interestingly, *FOXO1* mutations occurred exclusively in DH/TH cases (64% vs 0%, p = 0.004, Fisher's exact test), whilst *TP53* mutations mostly occurred in the cases with *MYC*-SH or *MYC*-amp (90% vs 18%, p = 0.002). Of the 17 patients that had *MYC* translocation and extra copies of the translocated *MYC* allele, 9 died within the first year of diagnosis and *TP53* mutation was associated with a poorer prognosis (p = 0.05). Our findings warrant more extended investigation of the clinicobiological impact of copy number gain of the translocated *MYC* allele, particularly in comparison to relevant cases without *MYC* copy number change.

Peripheral T-Cell Lymphoma, NOS with Aberrant Expression of CD20: Report of Two Cases

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Introduction: Peripheral T-cell lymphoma, NOS is a heterogeneous category of nodal and extranodal mature T-cell lymphomas that is associated with an aggressive clinical course. Aberrant expression of CD20 in PTCL is rare and the clinical and prognostic implications of this remain largely uncertain.
Case presentation: The first case concerned an 85 year old man who presented with a mediastinal mass. A bone marrow trephine showed heavy marrow infiltration by sheets of small to medium sized atypical lymphoid cells. Flow cytometry showed most of the cells were T cells that were positive for CD2, CD4, CD5 and CD56 with expression of CD20. CD7 was negative. Immunohistochemistry showed similar findings and the cells were also positive for CD20 in addition to expression of cytotoxic proteins TIA1, Granzyme B and perforin. CD79a and PAX5 were negative. The second case concerned an 80 year old man with widespread lymphadenopathy. A lymph node biopsy showed replacement of the architecture by a dense infiltrate of medium to large sized atypical lymphoid cells that surrounded numerous small collections of epitheloid histiocytes. Immunohistochemistry showed the lymphoid cells were BF1 positive T-cells co-expressing CD2, CD3, CD5, CD7 and cytotoxic proteins TIA1, Granzyme B and perforin. The cells showed variable aberrant expression of CD20. CD79a and PAX5 were largely negative. In both cases PCR showed clonal rearrangement of the TCR beta and gamma genes with no evidence of a B cell clone. The features of both were consistent with a peripheral T-cell lymphoma, NOS with aberrant expression of CD20.
Conclusions: CD20 expression in peripheral T cell lymphoma is rare and can be a diagnostic pitfall. Performing a wide panel of immunohistochemistry with flow cytometry and clonality studies is often needed to identify and correctly diagnose these cases. Aberrant expression of CD20 in these aggressive lymphomas can provide an additional therapeutic target for Rituximab.

Modelling Rhabdomyosarcoma Using the Chick Embryo Model

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma affecting children and adolescents. Localised disease has a 5-year survival rate of >70% following multimodal therapies, however patients with recurrent disease have a much worse overall survival. In other paediatric malignancies (neuroblastoma) adaptations to hypoxia have been shown to results in an aggressive cancer cell phenotype. The role of hypoxia in RMS is not as well understood and the purpose of this study is to investigate the role of hypoxia in RMS tumourigenesis with a view to develop novel therapeutics.
Methods: GFP labelled RD cells (embryonal RMS derived cell line, GFP-RD) were subject to in vitro hypoxic or normoxic environments. Cellular proliferation was assessed using sulforhodamine B (SRB) assay. HIF-1α expression, a marker of cellular adaptation to hypoxia, was analysed using western blotting. Hypoxic and normoxic pre-conditioned GFP-RD cells were transplanted onto the chick embryo chorioallantoic membrane (CAM) and tumour nodule forming efficiency (NFE), size and metastases were assessed using brightfield/ fluorescent imaging.
Results: SRB assays demonstrated greater cell proliferation in GFP-RD cells incubated for 24 hours in hypoxic vs. normoxic conditions. HIF-1α expression was greatest in GFP-RD cells incubated for 24 hours in hypoxic conditions. Both normoxic and hypoxic GFP-RD cells formed discreet nodules on the CAM but metastases were not seen. Hypoxic GFP-RD cells appeared to form larger nodules but their NFE was less than normoxic GFP-RD cells
Conclusion: HIF-1α may drive cell proliferation in hypoxic GFP-RD cells which subsequently form larger tumour nodules in the CAM model than normoxic cells but with less NFE. Future work will investigate these findings to further define the role of HIF-1α, angiogenesis and pattern of invasion.

Characterising the Response of a Metastatic Uveal Melanoma Cell Line to Hypoxia Using a Chick Embryo Model

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Uveal melanoma (UM) is a rare disease, but is the most common primary intraocular malignancy found in adults. It has an average incidence of 5 cases per million per year in the US and in Europe. Despite effective treatment of the primary tumour, metastatic disease will affect approximately 50% of UM patients, typically affecting the liver. A lack of adjuvant therapy leads to a poor prognosis for these patients and an urgent need for new therapies. Drivers of metastatic UM disease are poorly understood, however in other cancer types hypoxia is associated with enhanced tumour vascularisation and increased cancer cell survival in tumour microenvironments. This study investigates the role of hypoxia in UM and tumour progression, using the chorioallantoic membrane (CAM) assay in the embryonic chick model. The GFP-labelled metastatic UM cell line, MM66, was analysed for cell proliferation and HIF-1α expression in hypoxic (1% O_2) and normoxic (21% O_2) conditions. MM66 cells were grafted onto the CAM to analyse tumour nodule formation and metastasis using fluorescent imaging and histology. MM66 cells grown in hypoxia were found to have an increased rate of proliferation compared to normoxia. HIF-1α expression in hypoxic cells was greatest at 72 hours incubation and this was used to culture MM66 cells before implantation onto the CAM. Normoxic and hypoxic MM66 cells formed nodules on the CAM. This study was able to conclude that hypoxia is a driver for proliferation of MM66 cells *in vitro*. Both hypoxia- and normoxia-conditioned cells form tumour nodules on the chick CAM and further experiments will investigate the effects of hypoxia on tumour histology and metastatic development.

Distinguishing Neurosarcoidosis from Multiple Sclerosis Based on Cerebrospinal Fluid Analysis and Oligoclonal Bands: A Cohort Study

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Distinguishing neurosarcoidosis from MS can be troublesome, as definitive diagnosis requires invasive brain or meningeal biopsy demonstrating the characteristic granulomas. This study characterises a cohort of neurosarcoidosis patients with a focus on CSF analysis and whether this could help distinguish these two conditions.
This study enrolled 85 patients with a diagnosis of neurosarcoidosis based on stringent diagnostic criteria. The CSF protein, white cell count, and angiotensin converting enzyme levels were measured. The CSF and serum oligoclonal IgG patterns were compared. 80 patients had a probable or definitive diagnosis of neurosarcoidosis. The most frequent findings on MRI were leptomeningeal enhancement (35%) and white matter and spinal cord involvement (30% and 23%). CSF analysis frequently showed lymphocytosis (63%) and elevated protein (62%), but oligoclonal bands were rarely seen (3% in the CSF alone, and 11% matched in the CSF and serum). There was a lack of correlation between leptomeningeal involvement on imaging and CSF OCB. Serum ACE levels were elevated in 51% of patients, but in only 14% of those with isolated neurosarcoidosis. Large elevations in CSF protein, WCC and ACE occur in neurosarcoidosis, but are rare in MS. The diagnostic use of these tests is limited, however, since minimal changes may occur in both conditions. In contrast, intrathecal synthesis of oligoclonal IgG is a powerful discriminator as it is rare in neurosarcoidosis whilst occurring in 95–98% cases of MS. We suggest caution in making a diagnosis of neurosarcoidosis when intrathecal oligoclonal IgG synthesis is found.

Intra Cholecystic Papillary Adenoma Neoplasm of the Gallbladder (ICPN): Report of Two Cases

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Introduction: The definition of ICPN was first given by Adsay et al (Am J Surg Pathol. 2012 ;36(9):1279-301) for polypoid neoplasms arise in the gallbladder (GB). We report two cases of ICPNs.
Case reports:
Case 1: A 65-year old man showed a cystic/solid lesion in the gall bladder (GB) fundus. Microscopy of the GB wall showed chronic inflammation. The fundal lesion revealed an adenomymatosis containing foci of neoplasm comprising papillae lined by tall columnar cells with focal intracellular mucin; features mimicking the pancreatic counterpart lesion called papillary mucinous neoplasm (IPMN). Immunohistochemistry of the ICPN was positive for CK7, MUC1 (EMA) and MUC5AC. There was a focal positive staining with CDX2, CK20, MUC2, MUC4 and CEA. The Ki67 proliferative index was 1%.
Case 2: A 78-year old female with empyema of GB. At microscopy the GB showed severe acute inflammation. The mucosa of the GB showed papillary structures intermixed with solid and cribriform areas where cells had large nuclei. Immunohistochemistry of the papillary neoplasm was positive for CK7, MUC1, MUC4, CEA and MUC5AC. There was a focal positive staining with CDX2, CK20, and MUC2. The Ki67 index was 50%. The predominant cell lineage in these two cases was of biliary and gastric foveolar-type.
Conclusion: ICPN is an intramucosal GB mass showing dysplastic cells distinct from the neighbouring mucosa. ICPNs are pre-invasive neoplastic lesions. Dysplasia and carcinoma have been reported in adenomyomatosis but to the best of our knowledge, no cases of ICPN have been reported in adenomyomatosis of GB.

IgG4 Expression Correlates with Poor Prognosis in Pancreatic Adenocarcinoma

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Purpose of the study: IgG4, a member of the immunoglobulin family, has been implicated in the pathobiology of inflammatory disease (including autoimmune pancreatitis) and cancers. Its role in pancreatic cancers however remains to be elucidated. This study aimed to examine the role of IgG4 in pancreatic cancer, in both tumoural and inflammatory components.
Methods: The expression of IgG4 in pancreatic adenocarcinomas was studied by immuno-histochemical analysis of a tissue microarray (n=142) and analysed for clinicopathological correlations. The tumour inflammatory infiltrate, IgG4 positive lymphocytes (LCA stained) and plasma cells (CD138 stained) were investigated to understand their role in pancreatic malignancies.
Summary of results: Pancreatic tumour cells showed three positive patterns of staining: 47 (33%) golgi, 26 (18.3%) nuclear and 100 (70.4%) cytoplasmic. Significant positive correlations for tumoural IgG4 were observed with pancreatic cancer stage, grade, perineural invasion and recurrence ($p < 0.05$). IgG4 positive lymphocytic infiltrate was seen in 98 (69%) cases and presence at the tumour edge correlated with perineural invasion ($p= 0.03$). 80 (56%) cases exhibited an IgG4 positive plasma cell infiltrate which correlated with T stage ($p=0.02$) and vascular invasion ($p= 0.04$). Tumours with both strong golgi expression in tumour cells and high IgG4 positive lymphocytic infiltrate showed positive correlation with recurrence ($p=0.01$).
Conclusions: Overall, this study shows that IgG4, expressed in pancreatic tumour cells and its associated lymphoplasmacytic infiltrate, has significant correlations with poor prognostic features. Further exploration of the immunological milieu of pancreatic tumours will validate the functional significance of IgG4 associated immune networks in pancreatic malignancies.

Expression of Secretory Leukocyte Protease Inhibitor (SLPI) Detected by Immunohistochemistry in Hepatocellular and Cholangiocellular Tumours

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Introduction: Expression of secretory leukocyte protease inhibitor (SLPI) has been observed in hepatic macrophages and the biliary epithelium of livers affected by massive hepatic necrosis. In extrahepatic tumours SLPI has been considered to have a role as both promoter and inhibitor of cancer in different contexts. Here, we perform a preliminary investigation into the possible role of SLPI in the assessment of benign and malignant liver tumours.
Methods: We studied 10 cases of focal nodular hyperplasia (FNH), 15 large regenerative nodules (LRN), 11 dysplastic nodules (DN), 30 hepatocellular carcinomas (HCC), 17 combined hepatocellular cholangiocarcinomas (c-HCC-ChC), and 30 cholangiocarcinomas (ChC). The ChC cases included 10 intrahepatic, 13 hilar and 7 distal ChC. SLPI expression in both tumour tissue and background liver tissue was qualitatively assessed and characterised as negative, weak, moderate or strong.
Results: 98% of FNH, LRN, DN and untreated HCC showed no (55%) or weak (43%) expression of SLPI. Moderate to strong SLPI expression was observed in the cholangiocellular component of 47% of the c-HCC-ChC and in 73% of the ChC. Strong expression was a characteristic feature of the invasive component of all 13 hilar ChC, but was more variable in intrahepatic and distal ChC with strong expression in 50% and 57% of cases respectively. We observed that SLPI expression was prominent in the neoplastic epithelial component at interfaces with extralesional stroma and intralesional vascularised septa.
Conclusion: The preliminary results of this study indicate a role of SLPI in liver carcinogenesis, particularly in relation to cholangiocellular differentiation, and suggest that SLPI may have a role in the interactions between neoplastic epithelial cells and the adjacent stroma, possibly in terms of stromal invasion. Further studies are necessary to confirm these findings and investigate the role of SLPI in epithelial-stromal interactions in more detail.

An Unusual Solid Pancreatic Tail Lesion: Case Report

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Purpose of the study: To highlight the importance of cytological evaluation in diagnosis and treatment of unusual pancreatic masses.
Methods: A 67 year old lady with a diagnosis of breast cancer underwent staging CT which revealed a small hypervascular pancreatic tail lesion. FNA of the lesion showed a polymorphous lymphoid population, along with platelet aggregates, small capillaries and some spindle cells. No tingible body macrophages or germinal centres were seen. Immunostaining showed the lymphoid cells to be positive for CD45. CD8 was also positive highlighting cytotoxic T lymphocytes and splenic endothelial cells. Cam 5.2, Chromogranin and Synaptophysin were negative excluding a neuroendocrine tumour.
Results: The differential diagnosis lay between an intrapancreatic lymph node and intrapancreatic spleen (splenule), but the final diagnosis was a splenule, for which the patient required no further treatment.
Conclusions: Splenules are rare, benign lesions with excellent prognoses, which do not require surgical intervention. It is very important to be able to identify and definitively diagnose them cytologically with the use of appropriate immunostains in order to prevent unnecessary radiological or surgical intervention.

Lymph Node Yield, Nodal Status, and Excision Margin Status in Pancreaticoduodenectomy Specimens: A Seven-Year Audit Assessing the Impact of Preoperative Neo-Adjuvant Therapy

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Purpose: Pathological examination of tumour margin and lymph node dissection following pancreaticoduodenectomy (PD) for resectable pancreatic ductal adenocarcinoma (PDAC) is crucial in tumour staging and disease prognosis. The Irish National Cancer Control Program (NCCP) has set a minimum lymph node yield (LNY) of 10 as a key performance indicator (KPI) in PD specimens. In this seven year audit, we assessed adherence to the KPI and the effect of neo-adjuvant therapies (NAT) on LNY, nodal status and margin status in PD specimens of PDAC.
Methods: All pancreatic specimens at St. Vincent's University Hospital (SVUH) from January 2012 to December 2018 were retrieved via a SNOMED search of the laboratory information system (n=735). Margin status, nodal status, LNY, and pathological treatment response (PTR) data were obtained from pathology reports. Definition of negative margin status (R0) was based on the Royal College of Pathologists pancreatic cancer dataset. PTR was assessed using the College of American Pathologists (CAP) tumour regression grade (TRG).
Results: 183 PD specimens contained PDAC and NAT was administered to 50 patients. The mean LNY for NAT patients (14.02) was lower than for non-NAT patients (17.99). Overall, 160/183 (87%) of specimens achieved ≥10 LNY. Of the 133 non-NAT cases, 122 (92%) achieved a ≥10 LNY and of the 50 NAT cases, 38 (76%) achieved the same minimal LNY. R0 was achieved in 39/50 (78%) of NAT patients, and in 78/133 (59%) of non-NAT cases. Negative nodal status (N0) was achieved in 24/50 (48%) of NAT-patients and 36/133 (27%) of non-NAT cases. A favourable CAP TRG (0 or 1) was observed in 14/50 (28%) cases.
Conclusion: NAT has a negative impact on both LNY and adherence to the KPI. However, NAT has shown a positive impact on the R0 status and better outcomes in nodal status. Overall, adequate adherence to the KPI in PD specimens containing PDAC was met. Despite these findings, the majority of NAT cases did not demonstrate a favourable PTR.

Slides of Many Colours – Use of Tinctorial and Immunohistochemical Stains in Liver Biopsy Reporting: A Survey of the UK Liver Pathology Group and BDIAP 2018 Joint Liver Meeting Delegates

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Purpose of the study: To inform the RCPath Tissue Pathways for liver biopsies, we wanted to know current UK practice regarding the use of routine liver special stains and immunohistochemistry for medical and tumour biopsies.
Method: We compiled a SurveyMonkey questionnaire sent to delegates at the UKLPG/BDIAP Liver Meeting November 2018. This asked which tinctorial stains are used routinely for medical liver biopsies and which immunostains for hepatocellular lesions are available in their hospital.
Results: We received responses from 36 histology departments in the UK, with an additional 6 from Belgium and Holland. For medical liver biopsies in the UK the median routine stained sections was 9 (6–12). All but one include 2–4 H&E levels, along with an average of 6 (4–9) tinctorial stains. All centres routinely performed PASD, reticulin and Perls, and all but one do Shikata and/or Victoria Blue. All do one or more collagen stains, most often van Gieson (18/36). Nine routinely include an immunostain, usually K7. In Belgium and Holland, the number of stains is similar but choice varied – all use rhodanine and/or K7 instead of Shikata or Victoria Blue, and van Gieson is not used.
Immunohistochemistry: 20/42 departments receive resections for primary liver tumours. For antibodies used in the diagnosis of well differentiated hepatocellular lesions 15 have glutamine synthetase and Glypican 3 (with 13 having both). Other antibodies used for adenoma diagnosis are available in 5/17 UK centres and all 3 in Belgium/Holland. For 22 departments not receiving resections, HepPar1 and/or AFP are available in 21; glutamine synthetase and/or Glypican 3 are available in 2, sent away in 12, and never used in 6.
Conclusions: The study shows that routine use of tinctorial stains is ubiquitous among delegates at the meeting; liver biopsies require a high laboratory technical input. Immunostain use varies reflecting developments in diagnostic practice, and experience should be shared to ensure a common approach to diagnosis. Departments which are not liver surgical centres should have access to IHC or refer biopsies where the differential includes primary hepatocellular neoplasia.

Intrasplenic Epithelioid Malignant Mesothelioma: A Case Report

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Malignant mesothelioma (MM) most commonly arises from mesothelial cells lining the pleura, often related to asbestos exposure. Less commonly it involves the peritoneum and rarely pericardium or tunica vaginalis of testis or ovary. Localised, intraparenchymal MM in abdominal organs is very rare with few cases reported in liver, pancreas and spleen, likely having arisen from mesothelium of the capsule of the organs. A 75 year old lady presented with general malaise and a history of iron deficiency anaemia, hypertension and rheumatoid arthritis. Imaging revealed a 108mm splenic mass and a likely vascular liver mass. Tumour markers CA15-3, CA125, AFP and HCG were normal. Core biopsies of spleen lesion showed a malignant epithelioid tumour with few large bizarre nuclei, frequent mitoses and necrosis. A preliminary diagnosis of metastatic carcinoma of unknown primary origin was made based on positivity for cytokeratins AE1/3, 5 and 7. Positivity for Vimentin and WT1 was noted. Further tests revealed the tumour to be positive for Calretinin, D2-40, thrombomodulin and 34BetaE12 leading to a diagnosis of MM in the spleen. There was no known asbestos exposure. The patient underwent splenectomy for local symptom control. The resected spleen weighed 806grams and was 80-85% involved by tumour. Resection histology including targeted immunohistochemistry confirmed epithelioid MM. CT scan in Sep 2018 showed recurrence in splenic bed with peritoneal involvement. She unfortunately died 2.5 years after her diagnosis. Localised intra and perisplenic MM is very rare and may represent transformation of splenic mesothelial inclusion cysts or splenic capsular mesothelium. To the best of our knowledge this is only the second reported case of intrasplenic MM. Awareness of rare intrasplenic MM will facilitate correct diagnosis when posed with epithelioid tumours of unknown origin at pathological assessment of splenic tissue samples.

The Tale of Two Livers

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Introduction: The liver has a limited number of morphological changes that occurs secondary to pathological insults. In this poster, we present two classic but rare cases of liver biopsies.
Case Presentation 1: A 64 year old man of Asian origin, with a history of treated Hepatitis C, type 2 diabetes mellitus and seronegative arthropathy, was referred to Royal Surrey County Hospital with an isolated conjugated hyperbilirubinaemia. He developed a chest infection and his Liver Function Tests showed an elevated ALT of 90.
Biopsies were taken which showed steatosis, focal ballooning and prominent pericanalicular pigmentation,which was negative for Orcein and Perls staining. Features were suggestive of a hereditary cause of conjugated hyperbilirubinaemia. Urine coprophyrin studies are awaiting.
Case presentation 2: A 72 year old Caucasian retired lawyer presented to Royal Surrey County Hospital with weight loss and general malaise. CT thorax, abdomen and pelvis showed a 3cm hepatic mass in segment 8, which was radiologically consistent with a hepatocellular carcinoma. On further enquiry, he revealed a 30 year history of asthma.
Liver biopsy revealed PAS positive hyaline alpha-1-antitrypsin globules, mild-moderate chronic periportal inflammation and stage 3 fibrosis. A diagnosis of anti-1-anti-trypsin deficiency was made and upon review by the respiratory physicians, a pulmonary manifestation was also confirmed. PiZ genotype was confirmed on serology.
Discussion: Hereditary causes of conjugated hyperbilirubinaemia include Dubin-Johnson Syndrome and Rotor Syndrome, which cannot be distinguished morphologically, and require urine coproporphyins for definitive diagnosis. The diseases discussed are rare but have classical microscopic appearances that need to be correlated with the biochemical picture. Awareness of these entities amongst general histopathologists is instrumental in guiding the clinician towards the diagnosis.

Intra-Tumoural Expression of CD4 is Predictive of Recurrence in Non-Small Cell Lung Cancer Patients Managed with Surgery and Adjuvant Chemotherapy

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Purpose of the study: Non-small cell lung cancer (NSCLC) represents the majority of newly diagnosed lung cancers. Evading immune destruction has become one of the hallmarks of cancer and NSCLC has been at the forefront of therapeutic advances with immunotherapy. The immune system is implicated in the efficacy of several conventional chemotherapeutics. We wished to explore whether the adaptive immune response as reflected by immune biomarker expression could predict recurrence in patients with non-small cell lung cancer, and if so, whether the relationship was modulated by management with adjuvant chemotherapy.
Methods: Surgical resections of NSCLC from 220 patients were assessed in tissue microarray format for six immune biomarkers CD3, CD4, CD45RO, CD8, FOXP3 and ICOS. All biomarkers were assessed by digital image analysis using open source software (QuPath). Biomarker densities were dichotomised using ROC curves for survival analysis. All statistical analysis was performed using R.
Summary of results: No difference in immune biomarker expression was observed between patients who were managed with surgery and chemotherapy compared to surgery alone ($p>0.05$). Low CD4 expression was associated with disease recurrence ($p=0.05$). When stratified by treatment, low expression of CD4 in was found to be predictive of risk of recurrence in patients treated with adjuvant chemotherapy ($p<0.001$); patients with low intra-tumoural CD4 expression prior to treatment with adjuvant Cisplatin/Vinorelbine were more likely to recur than those with high CD4 expression.
Conclusions: Preliminary findings suggest that CD4 expression is predictive of recurrence in NSCLC patients managed with surgery and adjuvant chemotherapy using Cisplatin/Vinorelbine, but not surgery alone. Intra-tumoural CD4 expression could potentially be used as a biomarker to predict patients at high-risk of relapse when treated with adjuvant Cisplatin/Vinorelbine. Further work is required to validate these findings.

On-Demand EGFR Mutation Testing in Lung Cancer: Results in Three Hours

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Lung carcinoma is the most common cancer in the UK (excluding non-melanoma skin cancer) and the survival for these patients remains low. The majority present with non-small cell lung cancer (NSCLC) and around 16% of tumours have tyrosine kinase inhibitor sensitising mutations in the EGFR gene. These patients generally are often very sick and management decisions need to be made urgently. Pathologists are often under pressure to get results out in time for MDTs or clinics but not all centres have access to molecular diagnostics with fast turnaround times. Consequently, reflex testing of all patients is often employed, and this potentially wastes time and money. The Idylla™ EGFR Mutation Test offers rapid, on-demand results within three hours – from pathologist request to reportable result. The Idylla platform has shown promise in other mutation targets, including BRAF, KRAS and NRAS, and the potential for rapid results in lung cancer is attractive. This study aimed to assess the concordance of Idylla™ EGFR Mutation Test results with current standard tests. Forty formalin-fixed, paraffin-embedded NSCLC tumour cases (20 EGFR mutant and EGFR 20 wild type by standard testing) were retrospectively analysed by the Idylla™ EGFR Mutation Test (CE-IVD) and compared with PCR and NGS methodologies. The overall concordance between Idylla™ and standard testing was 92.5% (95% CI 80.14% to 97.42%) and the specificity of Idylla™ was 100% (95% CI 83.89% to 100%). The sensitivity was affected by loss of tumour content in tissue blocks in a small number of NGS cases; however, comparing Idylla™ with PCR alone, there was 100% concordance (95% CI 89.85% to 100%). The Idylla™ EGFR Mutation Test shows comparative accuracy to routine PCR testing for the most common EGFR mutations in NSCLC. The Idylla™ also offers very significantly reduced turn-around times compared with existing modalities and therefore could save money with eliminating the need for reflex testing.
This abstract has previously been published in full in Virchow's Archiv. https://www.ncbi.nlm.nih.gov/pubmed/30470932

Storage Cardiomyopathy: A Case of Sudden Death Due to Danon's Cardiomyopathy with Inflammation

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Purpose of the study: Cardiac disease is important in lysosomal glycogen storage diseases (Pompe and Danon disease), mucopolysaccharidoses and glycosphingolipidoses (Anderson-Fabry disease). The phenotype can vary to include hypertrophic and dilated cardiomyopathy, coronary artery disease and valvular disease. Danon's disease is a lysosomal storage disorder caused by an X-linked germline mutation in the LAMP2 gene resulting in cardioskeletal myopathy. Case of a 20-year-old female, previously diagnosed with Danon's disease with positive LAMP2 gene mutation and dilated cardiomyopathy who suffered a sudden cardiac death. We present the results of the heart examination.
Methods: The heart was examined using specific protocol with histology, selected immunohistochemistry and special stains.
Summary of results: There was cardiomegaly with biventricular dilatation and hypertrophy. On histology, hypertrophy and vacuolar degeneration of myocytes with patchy replacement fibrosis was identified. PAS staining failed to identify glycogen-containing lysosomes. Large epicardial areas contained a prominent CD3+ lymphocytic inflammation with myocyte necrosis.
Conclusions: Myocardial hypertrophy associated with vacuolar degeneration of myocytes and patchy fibrosis are hallmarks of Danon's cardiomyopathy. The absence of demonstrable intracytoplasmic glycogen should not discourage the diagnosis in the context of LAMP2 mutation. Genetics is important in the study of cardiomyopathies and LAMP2 mutations have an especially poor prognosis. The presence of an infiltrate of CD3+ lymphocytes has not been reported in Danon's cardiomyopathy before, and provides a potential explanation for her sudden death.

Chaperone-Mediated Autophagy Markers LAMP2A and HSC70 are Independent Adverse Prognostic Markers in Primary Resected Squamous Cell Carcinomas of the Lung

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Purpose of the study: LAMP2A and HSC70 are crucial players in chaperone-associated autophagy (CMA), a process of specific, targeted, lysosome-dependent degradation of proteins. CMA is crucial to maintain cell homeostasis and is frequently upregulated in cancer. Blockage of CMA may be therapeutically exploited. We aimed to evaluate the expression patterns and any prognostic significance of LAMP2A and HSC70 in pulmonary squamous cell carcinomas (pSQCC).
Methods: LAMP2A and HSC70 were analysed by immunohistochemistry in a consecutive cohort of 336 primary resected pulmonary squamous cell carcinomas using tissue microarrays (4 TMA cores from 2 different TMA blocks). Expression levels were determined by an immunoreactivity score (IRS) generated from the staining intensity and the percentage of positive tumour cells.
Summary of results: There was no significant intratumoural staining heterogeneity across the TMA cores. Moreover, no significant correlation between the two markers was seen. There was no association of marker expression with pathological parameters (pT category, pN category, TNM staging, grading). However, high LAMP2A and high HSC70 expression levels, defined as IRS levels above the 4th quartile, were associated with worse outcome, including overall survival (p=0.012 and p=0.001) and disease free survival (p=0.049 and p=0.036). Both markers were also independent adverse prognostic factors in multivariate analysis for overall survival (LAMP2A: HR=1.772; 95%CI 0.121-2.595; p=0.003; HSC70: HR=1.955; 95%CI 1.351-2.830; p<0.001) and disease free survival (LAMP2A: HR=1.528; 95%CI 1.066-2.191; p=0.021; HSC70: HR=1.482; 95%CI 1.047-2.098; p=0.027).
Conclusions: The CMA markers LAMP2A and HSC70 are variably expressed in pSQCC, and could be evaluated as predictive biomarkers for CMA-inhibiting therapy. High expression in untreated pSQCC presents an independent adverse prognostic factor.

Pulmonary Glomus Tumour

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A 33 year old woman presented with a chest infection to her GP. An abnormal chest x-ray prompted a chest CT which revealed a 20mm peripherally-located lesion in the left lower lobe. Following VATS wedge resection, frozen section and histology, a diagnosis of a low grade mesenchymal neoplasm with muscle differentiation favouring glomus tumour was made.
Introduction: Glomus tumours are uncommon tumours, predominately found in the dermis or subcutis of the upper and lower limbs. Although glomus tumours have been reported throughout the body, pulmonary glomus tumours are considered to be rare.
Case: The patient was referred to our Trust for a second opinion. MDT discussion led to VATS wedge resection and frozen section +/– proceed to lobectomy. It was impossible to provide a specific diagnosis on the frozen section and although tumour was confirmed, formal typing was deferred to paraffin sections. Completion lobectomy was not performed.
Histology: Macroscopically the lung wedge contained a 19mm firm tumour nodule with a greyish cut-surface. Microscopically the tumour was circumscribed and composed of cohesive rounded cells in nests and lobules. The cells were arranged around thin-walled sinusoidal vessels; had grooved and reniform nuclei, with occasional nuclear inclusions; moderate amounts of eosinophilic cytoplasm, which also showed clearing and micro-vacuolation; and prominent cell membranes. There was limited nuclear pleomorphism, no necrosis and no mitotic figures in 50hpf. Several rounds of immunohistochemistry were undertaken to identify the tumour, which showed positive staining with SMA, Caldesmon and Vimentin. All epithelial markers, neuroendocrine markers and vascular markers were negative, as was HMB45.
Conclusion: This is an example of a rare pulmonary tumour which posed an interesting challenge at frozen section and on histology due to the rarity of the lesion and its wide differential diagnosis.

IgG/IgG4 Staining Suggests Some Pulmonary Hyalinising Granulomas are Associated with IgG4-Related Sclerosing Disease

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Pulmonary hyalinising granuloma (PHG) is a rare condition of unknown aetiology, comprising circumscribed single or multiple nodules of thick hyalinised collagen bundles with chronic inflammation. PHG has been associated with immune disorders with one case report suggesting association with IgG4-related sclerosing disease. We therefore reviewed a cohort of PHGs, undertaking IgG and IgG4 immunohistochemistry, in order to assess whether there were features of IgG4-related disease (3 high-power-fields (HPF) were scored for absolute number of IgG4+ cells and IgG4+/IgG+ ratio). 8 cases of PHG (7 surgical and 1 core biopsy; 6 solitary and 2 multiples nodules) in 5 females and 3 males with an average age at diagnosis of 52 years (range 34-70) were assessed. All showed characteristic thick collagen bundles and a lymphoplasmacytic infiltrate. The highest determination of absolute numbers of IgG4-positive plasma cells/HPF in each case was: 37; 40; 49; 34; 155; 22; 83; 89 respectively (range 13-155). The average in IgG4/IgG ratio was 41% (64, 22, 46, 28, 49, 27, 44, 49 respectively). Thus, 3/8 (37.5%) of cases showed >20 IgG4-positive plasma cells and IgG4/IgG ratio greater than 40%, fulfilling criteria for IgG4-related sclerosing disease, this being 5/8 (62.4%) if using an IgG4/IgG ratio >40% only. Furthermore, these 5 cases had additional features adjacent to the PHG nodule comprising moderate to severe lymphoplasmacytic infiltrate with follicular hyperplasia. One case with high IgG4 scores showed coexistent non-necrotizing granulomas consistent with sarcoidosis. 3 cases had concomitant diseases as: rheumatoid arthritis, retroperitoneal fibrosis, pelvic sarcoma. However, only the first two showed >40% IgG4+/ IgG+ plasma cells but none greater than 50 IgG4 positive cells/HPF. These data suggest coexistent IgG4-related sclerosing disease in some cases of PHG. Staining for IgG4 and IgG, as well as the serum IgG4 levels, should be considered in all cases.

Impact of Provision of In-House *EGFR* Mutation Testing for NSCLC on Report Turnaround Times

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Purpose of the study: Our audit aimed to evaluate the impact of the introduction of internal *EGFR* mutation testing on report turnaround times, assess compliance with relevant international standards, and to compare local rates of *EGFR* mutations in non-small cell lung cancer (NSCLC) with national and international rates. Audit targets were set at 90% of *EGFR* tests to be reported within 7 days of request.
Methods: Data was collected retrospectively, with retrieval of records from the pathology electronic request system for all external *EGFR* test requests from July 2016 to December 2017, and for all internal *EGFR* requests from its launch in July 2017 to September 2018. Reporting dates and mutational status were collected from corresponding pathology reports. Timestamps on records were used to calculate turnaround times in working days. Data was analysed using chi-squared testing via Excel.
Summary of results: Internal *EGFR* testing resulted in a mean turnaround time of 5.8 days (±4, n= 179), as compared to 10.7 (±3.6, n=101) for externally performed *EGFR* tests. 83.2% of results for cases tested internally were reported within 7 working days, as opposed to only 5.0% of cases tested externally (p=<0.00001). 96.1% of internal *EGFR* test results versus 58.4% of external *EGFR* test results were available within 10 working days of request (p=<0.00001). The overall rate of NSCLCs harbouring *EGFR* mutations was 17.5%. Of those mutations, L858R was the most common (35.8%), followed by exon 19 deletions (24.5%). *EGFR* test failure rates were 4%.
Conclusions: In-house *EGFR* mutation testing for NSCLC samples has led to significantly reduced turnaround times compared to testing via external laboratories, but areas for improvement of service delivery have been highlighted. Our action plan includes a formal rota to ensure same day reporting and optimisation of our requesting program to flag overdue cases. Local mutation rates are in line with national and European reported rates.

A Filamentous Mimic of Lung Cancer

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Introduction: Actinomyces is a gram positive anaerobic filamentous bacterium, present in soil. There are 47 subtypes, 25 of which are found in humans. actinomyces israelii is a normal commensal of oropharynx, gastrointestinal tract, and urogenital tract.
Case: a 67 year old man with a history of bronchiectasis, presented with worsening symptoms. Base line blood tests were normal. A CT chest showed right lower lobe bronchiectasis, consolidation and a calcified right hilar lymph node. Bronchoscopy showed a right lower lobe endobronchial tumour and subsequent biopsy showed endobronchial actinomyces, associated with acute inflammation.
Discussion: Actinomycosis is usually non-pathogenic unless there is mucosal damage. Thoracic actinomycosis accounts for 15–20% of all the cases. Endobronchial actinomycosis is exceedingly rare. Since 1882, around 200 cases have been reported in the published literature. There are various clinical associations, such as broncholithiasis and intrapulmonary disease. Interestingly, it can also mimic TB and primary lung carcinoma resulting in misdiagnosis, which can occur in up to 25% of cases. Investigative radiological and endoscopic findings are usually non-specific but histology plays a vital role in an area of diagnostic difficulty due to its characteristic microscopic appearance and pattern of staining. Treatment includes prolonged course of beta lactam antibiotics or occasionally surgery. Despite its protracted course, the therapy is highly effective with a good prognosis and clinical outcome.
Conclusion: Our patient is currently on intravenous benzyl penicillin with symptomatic improvement. This case is a useful reminder to consider rare causes of endobronchial lesion.

An Unusual Dual Diagnosis of Rosai-Dorfman Disease Presenting Concurrently with IgG4 Related Disease

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Rosai-Dorfman disease (RDD) is a self-limiting, rare disease also known as sinus histiocytosis with massive lymphadenopathy. Currently classified under histiocytic disorders, the diagnostic histological hallmark of this disease is infiltration by large histiocytes (that express S100 and show emperipolesis) with plasmacytosis. The precise aetiology is not known. Recently, there have been some case reports that describe association of IgG4 related disease (IgG4RD) with RDD. We describe an unusual case of RDD presenting as a right atrial mass in a 52 year-old woman, which was excised by the cardiothoracic surgical team. Histology from the mass showed features typical of RDD – S100+/CD163+ histiocytes associated with emperipolesis with accompanying lymphocytes and plasma cells – along with an additional component: the presence of prominent fibrosis. The fibrosis replaced the myocardium and extended into the pericardium and endocardium. There was no necrosis. Immunohistochemical analysis showed one third of IgG-positive plasma cells also expressed IgG4. Thus, the overall morphology and immunophenotype was in keeping with a dual diagnosis of both RDD and IgG4RD. IgG4RD is typically characterised by a dense lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells associated with 'storiform' fibrosis. Cases of RDD demonstrating a local increase in IgG4 positive plasma cells are described in the literature, however, cardiac involvement by RDD is rare and the course is variable. Consensus guidelines on investigation and management of RDD have been published recently (Blood 2018: blood-2018-03-839753). The precise aetiopathogenetic link between RDD and IgG4RD is unclear. Due to the rarity of this disease, no case series exist till date. Further collaborative work is needed to investigate the co-existence of these diseases further.

Thymic Pathology: 20-Years' Experience at a Regional Cardiothoracic Centre

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Purpose of the study: Thymic tumours account for <1% of all neoplasms. These tumours have complex biology along with a relatively poorly characterised aetio-pathogenesis, making the study of these lesions important. This comprehensive review looks at all histologically confirmed thymic tumours reported at a regional cardiothoracic centre over a 20-year period.
Methods: All confirmed thymic pathology reports issued between 1997 and 2017 were retrospectively reviewed. Data was collected on the type of specimen received, the histological diagnosis and the concordance between biopsy and resection specimens.
Summary of results: 391 [100%] cases of histologically confirmed thymic pathology (resection specimens [71.9%], core needle and open biopsies [27.6%]) were identified during this period. Of these, 9.2% of cases were external referrals. 74.4% of the cases were neoplastic of which 78.0% were thymic epithelial neoplasms (thymoma, thymic carcinoma and neuroendocrine tumours). A resection specimen was received following a biopsy in 35.1% of cases. There was full concordance between biopsy and resection diagnoses in non-thymoma cases. In cases diagnosed as thymoma on the biopsy specimen, 50.0% showed full subtype concordance and 18.8% showed either a major or minor discordance in subtype correlation. In 31.3% of cases, correlation was not feasible.
Conclusion: This retrospective review highlights the wide spectrum of thymic pathology. The presentation emphasises importance of a good clinico-pathological correlation and an appreciation of the morphology of lesions at this site to appropriately triage often limited diagnostic biopsy material. Primary thymic neoplasms should always be considered within the differential diagnosis of a mediastinal lesion, including cases showing squamous or adenocarcinoma morphology.

Primary Pleural Epithelioid Haemangioendothelioma: A Case Report of a Rare Pleural Tumour

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Introduction: Epithelioid haemangioendothelioma is a rare malignant vascular neoplasm which can arise in soft tissue, bone, liver and lung but is extremely uncommon as a primary pleural tumour with less than 35 cases reported within the literature.
Case presentation: A 60 year old man presented with chest and shoulder pain and was found to have a unilateral pleural effusion, thought to be trauma related. He was otherwise fit and well, had never smoked and had previously worked in the armed forces. A follow up CT showed a hydropneumothorax with pleural thickening. A video-assisted procedure (VATS) was performed to drain the effusion and obtain a pleural biopsy. The biopsy showed thickened strips of pleura, diffusely infiltrated by cords and clusters of epithelioid and spindled cells. Focally these showed attempts at vasoformation with intracytoplasmic lumina, some of which were filled with red blood cells. Immunohistochemistry showed positive staining for Thrombomodulin, D2-40, CD31 and focally for CD34 and Pancytokeratin. They were negative for CK5, Calretinin and WT-1. RT-PCR showed the presence of a WWTR-CAMTA1 fusion, confirming the diagnosis of a primary pleural epithelioid haemangioendothelioma.
Discussion: Primary pleural epithelioid haemangioendothelioma is a rare, aggressive and chemotherapy insensitive tumour with a poor prognosis. It often presents with non-specific symptoms such as dyspnoea, chest pain and cough. Imaging often shows a pleural effusion with pleural thickening. It is important to be aware of this entity to avoid misdiagnosis as the radiological and histological appearances can mimic mesothelioma. Molecular analysis is a useful adjunct to diagnosing these tumours through demonstration of WWTR-CAMTA1 or YAP-TFE3 gene fusions.

Exploration of the Benefits of a Regional Digital Pathology Network for Referrals to a Tertiary Centre Specialist Melanoma Multidisciplinary Team Meeting

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Purpose of the study: To assess the existing pathway for referral of cases to a tertiary centre specialist melanoma multidisciplinary team (MDT) meeting for review of histology, evaluate timings of case movement and compare time differences between internal and external cases.
Methods: In order to assess the potential impact to a department as a whole and identify the cancer pathway, which could derive the most benefit from digitisation, volumes of outbound case referrals were obtained from four cellular pathology departments within the region, which referred cases into a central tertiary centre.
Timings of events in the patient diagnostic and management pathway were obtained from electronic patient records and the cellular pathology department laboratory information systems and used to calculate the overall pathway lengths for internal and external patients. Time differences for handovers between different processes within a hospital trust and for interprovider transfers were calculated.
Summary of results: Cases from external sites referred to the tertiary centre specialist melanoma MDT meeting passed through a number of additional steps between the initial histological diagnosis and the MDT review resulting in a lengthened pathway. For example, it took on average 6.6 days between the referral being recorded and the slides being received for review and 9.5 days between recording of the referral and the MDT date. In comparison, for internal cases the interval between recording of the referral and the MDT date was 5.5 days.
Conclusions: The use of whole slide imaging at the point of the initial diagnosis and development of a digital pathology network can reduce the time required for interprovider transfers for cancer cases. As preparation for an MDT often requires a fixed cut-off date for receipt of external cases, even a 6 day reduction in number of days in the pathway prior to the MDT (the delay from slide transportation) can potentially have marked impact for patients.

Post-Radiotherapy Cutaneous Mastocytosis: Once Seen, Would You Forget it?

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Cutaneous mastocytosis presenting during adulthood appears to endure for the duration of life. A large series has proposed that in all, and demonstrated that in most, of these cases patients have systemic mastocytosis with cutaneous involvement. The difference between prognosis in cutaneous mastocytosis and systemic mastocytosis renders the distinction in diagnosis and the choice of intervention important. We present the case of a 50-year-old female patient with a localised petechial rash in the left chest wall skin. The patient has a past history of breast cancer and had undergone left mastectomy and chest wall radiotherapy 15 months previously. A punch biopsy was performed. Microscopically the epidermis showed increased basal pigmentation, and an interstitial and perivascular loosely distributed infiltrate of mast cells and small numbers of eosinophils. The mast cells stain positively with C-kit. The appearance is of cutaneous mastocytosis. The patient has an elevated serum tryptase of 21.7ng/ml, suggesting systemic mastocytosis, although she has no systemic symptoms. Tissue was sent for KIT mutation testing, but unfortunately was incomplete as the percentage of nuclei estimated within the area of DNA extraction was below the limit of detection for Sanger sequencing. As a result a bone marrow biopsy is planned. A literature search has revealed only six cases of mastocytosis within irradiated areas, all of which occurred in the context of adjuvant radiotherapy for breast cancer. One of these cases is described as systemic mastocytosis. We explore clinical, histological and molecular genetic findings of this particular case, comparing them to those found within literature. We explore and consider the possibility of a Koebner effect secondary to radiotherapy, a neoplastic phenomenon or the possibility of these being cases of prior undiagnosed systemic mastocytosis.

Improving Turnaround Times and Cost-Effectiveness of BRAF Mutation Testing for Malignant Melanoma Using Immunohistochemistry and a Rapid PCR Platform

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Background: BRAF inhibitor drugs (1) are an important line of treatment for melanoma and require rapid testing of melanoma for BRAF mutations. We have investigated the feasibility and validity of using newly acquired immunohistochemistry (2) and a rapid PCR platform (Idylla) in detecting BRAF V600 mutations (3) within a large NHS hospital. Previously, the trust sent formalin fixed, paraffin-embedded melanoma samples to an external laboratory using Cobas 4800 PCR platform (gold-standard) for BRAF testing (1) with an average of a 7-day turnaround which dermatology found too long.
Method: The study included 37 melanoma samples tested by Cobas which we then tested with immunohistochemistry using the BRAF V600E antibody. It was feasible to also test 17 of these with Idylla which requires more tumour cells than Cobas.
Summary of results: Cobas PCR results were compared with results obtained from Idylla rapid PCR and immunohistochemistry. Immunohistochemistry picked up all but 2 of the positive results from Cobas and there were no false positives. All the results from Idylla matched with Cobas results. Turnaround time for immunohistochemistry is 24 hours whereby for Idylla is 2–3 hours although the latter performed 2–3 times per week. Immunohistochemistry is much cheaper (£26) compared to Cobas (£131) and Idylla (£112).
Conclusion: Immunohistochemistry and Idylla are more cost and time effective than sending to an external laboratory and a strategic algorithm has been designed for BRAF testing using immunohistochemistry followed by Idylla for immunohistochemistry-negative cases to pick up the remaining 5% false negatives (3).
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Angiomatoid Fibrous Histiocytoma: Report of a Rare Tumour at an Unusual Site and Review of the *EWSR1-CREB1* and *EWSR1-ATF1* Translocation Associated Neoplasms

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Purpose of the study: Angiomatoid Fibrous Histiocytoma (AFH) is a rare mesenchymal tumour arising in the subcutis of the extremities in children and young adults. AFHs harbour characteristic gene fusions involving the *EWSR1* gene (*EWSR1-CREB1*, *EWSR1-ATF1*) and *FUS-ATF1*, and belong to a family of translocation associated neoplasms. We present a case of AFH, highlighting diagnostic difficulties given the patient's age and the dermal location, and review the recent literature on this associated tumour family.
Methods: We retrospectively reviewed the histology, molecular features, and clinical records of a patient diagnosed with AFH at our institution, and undertook a review of recent literature on *EWSR1-CREB1* and *EWSR1-ATF1* associated tumours.
Summary of results: A 60 year old male presented with a one year history of a lump on the left ring finger. Histology demonstrated a dermal spindle cell tumour with a nodular and storiform pattern, with prominent pseudovascular spaces. Mitoses were readily apparent and cytological atypia with 'monster cells' was observed. The differential diagnosis included an aneurysmal fibrous histiocytoma vs AFH. Tumour cells expressed CD99, EMA and ALK. Break-apart FISH demonstrated a translocation involving *EWSR1* at 22q12. RT-PCR demonstrated an *EWSR1-CREB1* [t(2;22)(q34;q12)] gene fusion, confirming a diagnosis of AFH. This translocation is also found in tumours of both mesenchymal and epithelial origin.
Conclusion: This case of atypically presenting AFH highlights the value of molecular studies in establishing the correct diagnosis. An important pitfall is that many translocations are not tumour specific and are present in range of neoplasms with different therapeutic and prognostic implications. Correlation between the histology and molecular studies remains imperative. The mechanism by which common gene fusions can result in such a heterogeneous group of tumours is unclear, and warrants further study for potential therapeutic applications.

A Case of Multiple Storiform Collagenomas in a Patient with Cowden Syndrome

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Introduction: Storiform collagenoma, also known as sclerotic fibroma, is a rare benign neoplasm and is one of the lesser known cutaneous manifestations of Cowden Syndrome (CS). CS is due to a germline mutation in the PTEN gene and is characterised by an increased propensity to numerous benign and malignant tumours including hamartomatous gastrointestinal polyps, breast carcinoma and thyroid carcinoma. We present a case of multiple storiform collagenomas in a patient with CS.
Case History: A 62 year old male presented with a three week history of a flesh coloured, painless papule on the thigh. Past medical history included CS and clear cell renal cell carcinoma. On examination there were multiple firm lesions on the hands, thigh and face. Histology of each lesion revealed a well-circumscribed, unencapsulated dermal nodule composed of spindle cells admixed with hyalinised eosinophilic fibres arranged in a storiform architecture. The spindle cells were CD34(+), S100(–) and EMA(–). The clinical differential diagnosis included metastatic renal cell carcinoma. The histological differential diagnosis included storiform collagenoma, perineuroma, dermatofibroma and Pacinian neurofibroma. A diagnosis of storiform collagenoma was made and excision was curative.
Discussion: This case highlights the importance of clinicopathological correlation, being aware of the association of storiform collagenoma with CS and the potential wider implication of a diagnosis of storiform collagenoma. The previous history of renal cell carcinoma and extensive family history of malignancies at a young age should prompt one to consider a hereditary cancer syndrome. Although in this case the patient had a known diagnosis of CS, a skin manifestation is one of the commonest first signs of the condition and therefore you can potentially make a huge impact to the patient and their family by raising the possibility of Cowden syndrome.

A Case of Cutaneous Epithelioid Haemangioendothelioma Masquerading as a Sebaceous Cyst

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A 46 year old gentleman presented with a 2–3 year history of a lump on his scalp above the left ear. On examination this was a 2x2cm firm and tender lesion showing overlying erythema. Clinically the impression was of an inflamed sebaceous cyst which was subsequently excised. Macroscopically the specimen comprised an ellipse of skin with no obvious lesion seen on the surface. No cyst was identified and the cut surface showed a firm, pale appearance. On microscopy a well circumscribed lesion was present within the mid dermis extending into the subcutis. This was composed of sheets and cords of epithelioid cells with abundant pale eosinophilic cytoplasm, many of which showed an intracytoplasmic lumina containing a red blood cell. There was moderate cellular atypia with nucleolation, very occasional mitotic figures and a few apoptotic cells. The centre of the lesion showed a hyaline-myxoid matrix. On immunohistochemistry the cells were strongly and diffusely positive for CD31 with patchy staining for CD34. SMA and EMA were negative with very weak but diffuse staining for cytokeratin MNF116. The MIB1 proliferation index was very low. Overall the morphological and immunohistochemical features were consistent with a lesion of endothelial cell origin and given the lack of lobular architecture and inflammation but presence of moderate atypia was diagnosed as an epithelioid haemangioendothelioma. The patient is undergoing further radiological investigation to assess for multisystem involvement. Epithelioid haemangioendothelioma is a vascular neoplasm with behaviour lying between an epithelioid haemangioma and angiosarcoma. The majority arise within soft tissues and visceral organs such as the lung and liver. Solely cutaneous presentations are rare, usually found on the extremities and can be mistaken for benign entities clinically. Although deemed low grade these tumours have a potential for metastatic behaviour, however where resectable have a better clinical prognosis.

HP1BP3 Expression and its Significance as a Prognostic Marker in Human Primary and Metastatic Malignant Melanoma

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Melanoma is the deadliest form of skin cancer, accounting for 80% of all skin cancer related deaths. As such, research into novel proteins as potential prognostic indicators is vital. Despite the necessity, clinical relevance of these so far has been debatable. Epigenetic regulators, such as proteins involved in maintaining chromatin structure or regulating the cell cycle have been of particular interest. HP1BP3, a member of the H1 linker histone family, is one example of these. This study was conducted to investigate the association between HP1BP3 expression and survival of primary and metastatic melanoma patients. To determine HP1BP3 expression in patient melanoma samples, immunohistochemistry (IHC) was used to stain TMA slides, consisting of primary (n=451) and metastatic (n=448) cases. Tumour cores were scored using the H-score method, accounting for percentage positivity and intensity of staining in the nuclei of melanoma cells. High expression of HP1BP3 was associated with higher recurrence-free survival rate in primary melanoma patients($p= 0.04^*$) which was confirmed by multivariate analysis. High HP1BP3 expression in primary melanoma cases was also associated with lower mitotic rate ($p= 0.04^*$) and focal tumour infiltrating lymphocytes (TILs), ($p= 0.04^*$). Trends were seen between high HP1BP3 expression and overall survival ($p= 0.08$) and absence of a BRAF mutation ($p= 0.05$) in the primary cohort. No other significant associations were found. These findings suggest that high HP1BP3 expression is an independent prognostic factor, predicting longer recurrence-free survival in primary melanoma patients. This result, and the associations between HP1BP3 and known prognostic factors, necessitate further research into the role of HP1BP3 in melanoma.

Detection of the BRAF V600E Mutation in Primary and Metastatic Malignant Melanoma: An Evaluation of the Immunohistochemical Approach

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The BRAF V600E mutation is found in over half of melanomas, making it an attractive therapeutic target; this led to the development of anti-BRAF drugs. Detection of this mutation is currently performed using pyrosequencing. A monoclonal antibody specific for the BRAF V600E mutant protein has been developed, thus immunohistochemistry (IHC) can be used for detection. The primary aim is to evaluate IHC as a method of BRAF V600E detection compared to pyrosequencing. Secondary aims include analysing clinicopathological and survival data for primary melanoma cases with comparison to their BRAF V600E mutation status. After collecting the pyrosequencing data from patient records, 5 primary and 4 metastatic melanoma tissue microarrays were stained using the locked down protocol installed on the Roche Ventana Benchmark Ultra platform. The anti-BRAF V600E (VE1) mouse monoclonal antibody (Roche) was used. Informed consent from patients was obtained for their tissue to be used and stored under the Nottingham Health Science Biobank. Slides were scored using the H-scoring method and verified by a pathologist. SPSS v24.0 was used for statistical analysis. Sensitivity and specificity of the antibody compared to pyrosequencing were deduced for the primary cohort (65.91% and 91.94% respectively) and the metastatic cohort (86.59% and 98.35% respectively). BRAF mutation status was significantly associated with age and site ($p<0.001$), ulceration ($p=0.027$), mitosis ($p=0.011$), tumour-infiltrating lymphocytes ($p=0.042$), and histological subtype ($p=0.013$). There were no significant associations between survival and BRAF V600E status. The clinicopathological prognostic factors provide evidence for the BRAF V600E mutation as a positive prognostic indicator. IHC could be used to screen for BRAF V600E mutations, with negative cases being referred for molecular testing like pyrosequencing. This would ensure the detection of other BRAF mutations that could benefit from anti-BRAF drugs.

An Unusual Case of Hyperkeratosis

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Hyperkeratosis lenticularis perstans (Flegel's Disease) is a rare, hyperkeratotic skin disorder which typically presents on the lower extremities of Caucasian middle-aged patients. Most cases are sporadic although familial cases with an autosomal dominant mode of inheritance have been reported. Clinically the condition mimics many other hyperkeratotic and inflammatory disorders and the diagnosis is only confirmed on histopathological and clinical correlation. The condition manifests with asymptomatic keratotic/scaly red/brown papules which histomorphologically show lamellar hyperkeratosis with abrupt peripheral basket-weave orthokeratosis, irregular acanthosis and underlying lichenoid lymphocytic infiltrate. The pathogenesis is unknown although ultraviolet light and cell-mediated cytotoxicity against epidermal cells have been implicated. Herein we describe an unusual case of hyperkeratosis in a 44 year old female who was referred with numerous light brown papules on both her upper and lower extremities and neck. A punch biopsy revealed skin containing a central area with abrupt compact hyperkeratosis and focal parakeratosis, epidermal thinning, interface dermatitis with occasional colloid bodies and underlying lichenoid chronic inflammation. The clinical and histomorphological appearances were in keeping with hyperkeratosis lenticularis perstans. There was no evidence of dysplasia or malignancy.

Audit of Radical Lymph Node Dissection Involvement by Metastatic Melanoma in Patients with a Positive Sentinel Lymph Node

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Introduction: The Scottish Intercollegiate Guidelines Network (SIGN) guideline for melanoma advises that radical lymph node dissection (RLND) is indicated in patients with metastatic melanoma in a sentinel lymph node (SLN). RLND carries a risk of significant morbidity and anecdotal evidence suggests that most patients with a positive SLN have a negative RLND. This audit analysed whether there are characteristics of the primary melanoma, the SLN, or the patient that are associated with having a negative RLND.
Methods: A retrospective analysis was performed of anonymised data from all patients who had melanoma, a positive SLN, and subsequent RLND between 2006 and 2018 ($n = 38$) in our centre. Information regarding the primary melanoma and the RLND was extracted from reports. The slides of the SLNs were reviewed by a consultant dermatopathologist and data regarding tumour burden (1mm or less vs greater than 1mm, subcapsular vs parenchymal) were recorded. The data were analysed using SPSS Statistics.
Results: 66% of patients with a positive SLN had a negative RLND. 88% of patients with subcapsular metastatic melanoma only in their SLN and 79% of patients with metastatic melanoma measuring 1mm or less in their SLN had a negative RLND. The mean Breslow thickness of the primary melanoma in patients with a negative RLND was slightly less than that for patients with a positive RLND (3.11mm vs 4.99mm, $p = 0.04$). The mean age of patients with a negative RLND was significantly lower than that of patients with a positive RLND (47 vs 63, $p < 0.002$).
Conclusion: Factors such as SLN tumour burden, pT stage of the primary melanoma or the patient's demographic might help predict which patients are likely to have a negative RLND and could therefore spare them the associated morbidity. This would also reduce the medical, surgical and pathological resources required in managing these patients.

Benign Hidradenoma Metastasising to Regional Lymph Node: A Case Report of a Rare Entity

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Nodular hidradenoma is a benign adnexal tumour of sweat gland origin that usually presents as a solitary solid or cystic nodule in the skin. The course is usually benign with no loco regional recurrence or distant metastasis. However rare cases of hidradenoma with benign histology but showing vascular invasion have been reported in literature. Even rarer are benign hidradenoma with regional lymph node metastasis which to the best of our knowledge, only three cases have been reported so far. We hereby report a forty nine year old gentleman who presented clinically with a 1.5cm intact cyst in the right shin. On excisions biopsy, this was a dermal based tumour with ductal differentiation exhibiting histological features of a benign nodular hidradenoma with no atypical features. However an excision biopsy of a right groin lymph node that was simultaneously submitted contained a deposit of nodular hidradenoma. This again did not show any atypical features other than it was a "metastasis" in the lymph node draining the dermal benign hidradenoma. This was later confirmed to be a "benign metastasis" of nodular hidradenoma after expert consultation. "Benign" metastasis of nodular hidradenoma to the lymph nodes is a challenging entity that needs to be recognized by the pathologists. It is probably prudent to pursue a long term follow up in these patients to confirm that the course is benign. The few similar cases that have been reported so far in the literature have shown an uneventful long term follow up. In our case, the lesion in the skin was completely excised and long-term clinical follow up was recommended.

Audit of Dysplastic Naevi in a Tertiary Referral Centre

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Dysplastic naevi (DN) are defined as atypical clinically and/or histologically by architectural and cytological atypia. They were first reported in 1987 by Clark, Lynch and Elder as histologically defined lesions in melanoma-prone families. Despite the multiple consensus conferences and studies with regard to their diagnosis, guidelines for diagnosis and management including wider excision remain unclear. In this audit, we reviewed the diagnosis of DN in a tertiary referral centre over a one year period with a 6 year follow up to look at the burden of DN within our service and the excisional status of these naevi. All DN diagnosed in 2012 were retrieved from the pathology files at our institution with a 6 year follow up. DN accounted for 19.3% (425/2205) of melanocytic lesions diagnosed with 79.1% of those diagnosed as mildly DN. Moderately DN accounted for 14.6% and 5.2% were severely DN. Of these, 407 cases were classified as excisions while the remaining 18 were diagnostic biopsies. Of 407 excisions, 302 were documented as being completely excised while a further 99 had positive or close margins (defined as ≤1mm to margin). Of these 99 cases, only 17 (17.2%) had a re-excision, 7/17 (41.2%) of these being mildly dysplastic, 4/17 (23.5%) moderately dysplastic and 6/17 (35.3%) severely dysplastic. Of those cases that did not have a re-excision, the majority (55/76, 72.4%) were mildly DN with 3 severely DN. No recurrence or new naevi in the vicinity were documented during the 6 year follow up in 69/76 cases while the remaining 7 had a DN diagnosed in the vicinity. DN account for a significant proportion of melanocytic lesions submitted for histopathological examination, the majority of which are mildly dysplastic. Despite only 17.2% of cases with positive or close margins on initial excision having a re-excision, no definite recurrence of a dysplastic naevus was reported in our series suggesting that wider excisions of dysplastic naevi are not always necessary.

The Role of FOS Expression in the Diagnosis of Bone-Forming Tumours: Osteoid Osteoma / Osteoblastoma and Osteosarcoma

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Purpose of the study: Osteoblastoma and osteoid osteoma are together the most frequent benign bone-forming tumour, arbitrarily separated by size: the former being more than 2cm in greater dimension. Although in most clinical scenarios these are straight forward diagnoses, in some instances, it can be very difficult to differentiate osteoblastoma from osteosarcoma, in particular the osteoblastoma-like variant. Following our group description of FOS gene rearrangement in these tumours, the aim of this study is to evaluate the value of immunohistochemistry in osteoid osteoma, osteoblastoma and osteosarcoma for diagnostic purposes.
Methods: Spinal and non-spinal osteoblastomas (n=83), osteoid osteomas (n=33) and sequential biopsies of osteosarcomas (n=215) were retrieved from the files of the Royal National Orthopaedic Hospital and Basel University Hospital. A total of 332 cases were tested with antibodies against cFOS.
Results: 83% of osteoblastomas and 73% of osteoid osteoma showed significant expression of cFOS in the osteoblastic cell tumour component. Of the 19 cases negative for cFOS expression, 4 showed FOS gene rearrangement by FISH and 12 were non-informative for FOS and FOSB FISH. No additional cases of FOSB rearranged were identified. Of the osteosarcomas, 10% showed cFOS expression, usually focal and in highly atypical areas. 4% of the cases showed more conspicuous expression.
Conclusions: FOS rearrangement is the genetic abnormality underpinning osteoblastomas and osteoid osteoma. cFOS immunohistochemistry is positive in the vast majority of such cases. Expression, usually focal or patchy, is seen in up to 14% of osteosarcoma biopsies. In most osteosarcomas the expression is seen in highly atypical, non-osteoblastic areas, which would not be included in histological differential diagnosis. Our findings highlight the importance of undertaking assessment of expression patterns of antibodies in the light of morphological, clinical and radiological patterns.

Expression of Hormone Receptors and PARP-1 in Aggressive Fibromatosis

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Purpose of the study: Aggressive Fibromatosis or desmoid tumour, is a benign, but locally invasive entity. State-of-the-art treatment is mostly radical excision. However, incomplete surgery leads to a high risk of recurrence. Hormone modifying therapies were reported to be successful in several cases but need additional evaluation. The DNA-repairing enzyme Poly ADP Ribose Polymerase-1 (PARP-1) might contain therapeutic potential, suggested by successful trials of PARP-inhibition in other malignancies, especially in carcinomas and selected sarcomas.
Methods: In this study, we retrospectively investigated the expression of the hormone receptors: estrogen receptors (ER) α and β, progesterone receptor (PR) and androgen receptor (AR), as well as PARP-1 by immunohistochemistry and quantitative RT-PCR in tissue samples of Aggressive Fibromatosis (n=69). Immunoreactivity scores were employed to quantify staining status. PCR-results were numerically analysed as well as using explorative cutoffs. The obtained expression patterns were correlated with clinical-pathological parameters in order to detect prognostic factors.
Summary of results: The analysed hormone receptors showed mostly no reactivity to immunohistochemical staining. PARP-1 on the other hand exposed variable nuclear positivity in all stained samples. Univariate survival analysis portrayed higher ERα expression to be a negative prognostic factor (p=0.005). Multivariate analysis demonstrated that higher PARP-1 expression is associated with earlier relapse (p=0.003). In general, survival analyses underlined that recurrent tumours relapse faster than primary tumours (p<0.001).
Conclusions: According to this study, PARP-1 expression is associated with poorer prognosis, i.e. faster tumour recurrence. PARP-1 expression could therefore be an interesting target for a new and rather personalized treatment. Hormone receptor status has limited prognostic value in our study.

Proximal-Type Epithelioid Sarcoma: Case Report of a Rare Aggressive Tumour

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Proximal-type epithelioid sarcoma is a rare aggressive tumour. In contrast to the classic-type, it occurs in an older age group and at more proximal sites. It is associated with frequent recurrences, early metastasis and a high mortality. Definitive diagnosis relies on histological examination. Early detection, complete surgical excision +/– adjuvant therapy and close follow-up remain the mainstay of treatment. We report a case of a 49 year old male presenting with a lump in the left groin. An initial diagnostic biopsy suggested a high grade malignancy with possible origin from the urinary bladder in view of the GATA3 positivity. However clinically there was no bladder lesion. Imaging confirmed a 106mm soft tissue mass within the lower abdominal wall. The excision specimen showed a 90mm tumour in the deep soft tissues. Histology revealed epithelioid cells with rhabdoid morphology which led us to consider a wide range of differential diagnosis including metastatic poorly differentiated carcinoma, melanoma, leiomyosarcoma, rhabdomyosarcoma, angiosarcoma and anaplastic large cell lymphoma. A wide panel of immunohistochemical markers showed positivity for AE1/AE3, EMA, Vimentin, GATA3 and loss of SMARCB1 (INI1) in the tumour. A diagnosis of proximal-type epithelioid sarcoma was made. Material sent for cytogenetics showed bi-allelic inactivation of SMARCB1 (INI1) consistent with epithelioid sarcoma. The explanation for GATA3 positivity remained unclear. The patient on follow-up had recurrent disease with widespread systemic and nodal metastasis, ultimately resulting in patient's death. We report this rare sarcoma with a wide clinical and histological differential diagnoses. We want to highlight the pitfalls caused by GATA3 positivity which initially resulted in unwarranted investigations for a bladder primary. Pathological diagnosis is vital for early recognition and timely management with close follow-up in view of its poor prognosis.

Intestinal Secretory Leukocyte Protease Inhibitor (SLPI) is Induced by Repetitive Microbial Contact and is Increased in a Subgroup of Inflammatory Bowel Disease (IBD) Patients

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IBD patients are a heterogeneous group with varying therapy responsiveness. One cause for this variation may be the dual role for innate immune defects. Patients may have loss of control of the innate immune system, causing hyperactive immune responses to harmless bacteria. Alternatively, patients may have hyporesponsive immune function, resulting in failure of microbial eradication. Strikingly, methods identifying these patient groups are lacking as these immune processes are not fully elucidated. Recently, we have observed that intestinal SLPI expression is driven by repetitive microbial interaction. SLPI inhibits NF-κB activation and reduces host responses to harmless bacteria. Therefore, we hypothesized that high intestinal SLPI expression identifies IBD patients with insufficient anti-microbial activity. We analysed 84 biopsies from therapy-naïve paediatric IBD patients by quantitative PCR and observed a 10-100 fold increased SLPI mRNA expression in macroscopically inflamed ileac and colonic biopsies compared to non-inflamed biopsies. Next, we analysed SLPI protein expression by immunohistochemistry in 57 biopsies from paediatric IBD patients. Ileac SLPI protein expression was low, but colonic SLPI was increased in approximately half of the IBD patients. To assess whether high SLPI correlates with insufficient anti-microbial activity, we compared biopsies from a chronic granulomatous disease (CGD) patient (defective microbial killing) to an interleukin-10 receptor alpha (IL10RA) deficiency patient (hyperactive immune system) and found high SLPI protein expression in the CGD patient but nearly absent expression in the IL10RA deficiency patient. Our data suggests that high intestinal SLPI expression identifies a subtype of IBD possibly reflecting insufficient anti-microbial activity. To demonstrate this, we are currently investigating how intestinal SLPI expression relates to antimicrobial antibody levels and therapy responses in a larger therapy-naïve IBD cohort.

Combining RNAscope and IHC results to Identify a C-MET Aberrant High-Risk Colorectal Cancer Patient Subgroup with a High RNA and Low Protein Expression Profile

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Colorectal cancer (CRC) is Europe-wide the second most common cause of cancer death and represents with several different phenotypes. c-MET overexpression is associated with poor outcome and c-MET inhibitors were suggested to be applicable to resistant tumours. Despite promising results in clinical phase 1 and 2 trials, phase 3 trials tend to be unsuccessful. A reason for the latter could be the lack of an officially regulated and well-established scoring system for c-MET. The aim of this study was to analyse c-MET DNA amplification as well as to score RNA and protein levels, correlate these results, evaluate them statistically and identify the prognostic significance of aberrations. The present study includes 241 FFPE tissue microarrays (TMAs) of CRC patients that were stained during double-DNA in-situ hybridization (DDISH), RNAscope processing and immunohistochemistry (IHC). The samples were evaluated by digital pathology methods. We show for the first time that a high-risk c-MET aberrant subgroup must be identified by a combination of RNA and protein evaluating methods. Furthermore, our results suggest that the high-risk patients (HR = 2.1, 95% CI = 1.2 - 3.67) that present with a 35 % lower 5-year survival, display a phenotype comprised of high RNA levels and low protein expression. This finding contrasts with earlier studies that correlate protein overexpression with poor prognosis but are in line with recent study findings from 2016. It is crucial to identify the biological mechanism behind this phenotype to establish appropriate treatment options. Furthermore, we suggest to conduct a more detailed analysis concentrating on focal and invasive edge expression of c-MET.

The Molecular Aspects of Aberrant Negative P53 Immunohistochemistry in Barrett's Oesophagus: A Pilot Study

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Purpose: p53 immunohistochemistry is an adjunct in the histological diagnosis of dysplasia in Barrett's oesophagus (BO). Previously p53 immunohistochemistry assessment was binary with recognition of aberrant positive or normal staining. Currently an aberrant negative/null staining pattern is also recognised, which results in total loss of staining compared to background wild-type/normal staining and may carry a greater risk of progression to adenocarcinoma. This pilot study aims to determine the molecular biology of aberrant negative p53 BO-related dysplasia using Next Generation sequencing (NGS) in endoscopic biopsies and assess if mutations can also be detected in non-dysplastic BO.
Methods: Biopsies showing BO-related dysplasia with aberrant negative p53 immunostaining were identified from archives and reviewed for suitability. Tissue was macrodissected from annotated sections, with separate dissection of dysplastic and non-dysplastic tissue. DNA was extracted and analysed using NGS (Illumina MiSeq) and Sanger sequencing.
Results: 12 cases were identified for this pilot. 3 cases provided separate samples of dysplastic and non-dysplastic areas. 44 TP53 mutations were detected in 9/12 dysplastic samples and 3/3 non-dysplastic samples. The most common mutations seen were missense (61%) with exon 4 most frequently affected (61%). Sanger sequencing on 4 cases did not detect any mutations.
Conclusions: NGS has characterised TP53 mutations in biopsies showing BO-related dysplasia associated with aberrant negative p53 immunostaining and may be more sensitive than Sanger sequencing. No single defining mutation was identified but missense mutations in exon 4 were most frequent. NGS also identified mutations in separate non-dysplastic BO. Further studies can be expanded to include cases BO-related dysplasia associated with wild type and aberrant positive p53 staining and non-dysplastic BO. Such studies may help to establish a role for NGS in future surveillance strategies.

Cten Ability to Induce Migration is Independent of its Nuclear Localization

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Cten (Tns4) is a member of the tensin family and it is not expressed in normal colorectal tissue. However, in Colorectal cancer it is upregulated where it acts as an oncogene and enhances EMT, migration and invasion but not proliferation. The structure of Cten has a predicted Nuclear localization signal (NLS).NLS tags Cten protein to be imported to the nucleus. In abnormal situations like cancer for example, many proteins translocate to the nucleus and enhance migration, invasion and proliferation. In colorectal cancer, Cten is mainly expressed in the cytoplasm, however, it has been reported in that it translocates to the nucleus in several colorectal cancer cell lines and patient tissues. The translocation to the nucleus usually requires Nuclear Localization Signal (NLS) within the protein sequence. These amino acid sequence direct proteins to be translocated to the nucleus through binding to Importin. There is speculation about the role of Cten in the nucleus in colorectal cancer, however the exact biological function of Cten localization in the nucleus is to be investigated. We have used NLSMapper website to predict the nuclear localization signal of Cten. The predicted NLS was then deleted from the wild-type Cten plasmid using Site-directed mutagenesis. We then transfected HCT116 with Empty vector, wild-type Cten, and Cten(Δ NLS). This was followed by western blot, proliferation and Migration assay. Western blot and immunofluorescence data shows that deleting the predicted NLS prevented Cten protein from translocating to the nucleus. Cten induces migration but not proliferation in HCT116. The ability of Cten to induce migration was retained when we prevented the nuclear localization of Cten.
Conclusion: Cten translocation into nucleus was prevented by deleting NLS.
The ability of Cten to induce migration in colorectal cancer is independent of its nuclear localisation.

Validating a Tissue Microarray Linked Colorectal Cancer Cohort

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Introduction: Tissue microarrays (TMA) offer an important way of testing the predictive and prognostic power of novel biomarkers. In order to be a useful biomarker assay, the TMA should be linked to a cohort of diagnostic and follow-up data. These data should be valid – one way of ensuring this is to demonstrate concordance with other published cohorts so that the conclusions are generally applicable. Here we test the validity of our new colorectal cancer cohort.
Methods: We identified 1000 eligible cases of colorectal cancer from our local pathology database, including full pathology reports of surgical biopsies and resections (initial diagnosis from 2008 to 2014). Patient demographics and follow-up data were gathered from the hospital patient information system. We investigated a range of known risk factors and their effects on overall survival, time to recurrence and cancer-related mortality.
Results: The average age was 68.8 (SD: 11.37) and 56.8% of patients were male. Mean follow-up time was 54 months (SD: 29). 88.6% were moderately differentiated (2% well-differentiated, 9.3% poor); the overall stage distribution was 1 – 16%, 2 – 40.1%, 3 – 32%, 4 – 11.9%. A range of established clinical (eg. overall stage, metastases) and pathological (eg. vascular/perineural invasion, T3/T4 stage, high lymph node stage) risk factors were associated with adverse prognosis (overall survival, cancer-related mortality, recurrence). We confirmed that for high tumour stage cancers, lymph node status has minimal further impact on prognosis.
Conclusion: We have constructed a valid colorectal cancer cohort which underlies our new tissue microarray. This will be an invaluable resource for investigating novel colorectal cancer biomarkers.

Investigating the Role of TNS4 in the Colorectal Tumour Microenvironment Using 3D Spheroid Models of Invasion

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TNS4 (Tensin 4 or Cten) has been identified as a putative oncogene in colorectal cancer (CRC) contributing to the dynamics of cell adhesion, motility, invasion and epithelial to mesenchymal transition, as shown by vitro 2D-based assays. Our objective was to assess the role of TNS4 in 3D spheroid proliferation and invasion into the extracellular matrix. In order to mimic the 3D tumour microenvironment in vitro we used a spheroid model combining cancer-associated fibroblasts (CAFs) and TdTomato transduced colorectal cancer cell lines where TNS4 was stably knocked down via lentiviral transduction with PLKO.1 shTNS4. Cell growth was measured by spheroid volumetry, TdTomato fluorescence and 3D and quantification of DQ-BSA green degradation marker in the extracellular matrix was used as a measure of invasion. For the invasion assay, spheroids were embedded in basal membrane extract containing DQ-BSA green and overlaid by media containing EGF (40ng/mL) as a chemoattractant. Spheroids were imaged by confocal microscopy Z-stacks after 96h of invasion and the volume of emitted DQ-BSA green and TdTomato fluorescence was measured. TNS4 knockdown increased cell proliferation slightly, but significantly in cell lines producing compact spheroids, and also reduced adhesivity between CRC cells and CAFs or collagen type I. In general the addition of CAFs in spheroids supports proliferation of CRC cells, whereas CAFs themselves do not proliferate in low adherence conditions. The 3D invasion assay has shown a reduction of invasion with TNS4 knockdown, whereas addition of CAFs to spheroids increased the extracellular matrix degradation. In a 3D spheroid model, TNS4 seems to have an effect in modulating the ability of CRC cells to invade the extracellular matrix and their proliferation. These results confirm previous research using 2D-based assays and support the role of TNS4 as an oncogene in CRC, suggesting its value as an actionable therapeutic target to prevent early metastasis.

Preliminary Report of TNS4 Overexpression in the ApcMin Mouse Model of Colorectal Cancer

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ApcMin mice constitute a gold standard experimental model of colorectal cancer, mimicking the familial adenomatosis polyposis (FAP) disease in humans. Tns4 is part of the tensin family, localized in focal adhesions and is considered a putative oncogene in colorectal cancer. Tns4 is overexpressed in inflammatory bowel disease and human colorectal tumours, from the earlier stages of adenoma development. Our objective was to assess expression of Tns4 in early stage adenomas developed in ApcMin mice. Samples were collected to RNA later from polyps and adjacent normal intestine of sacrificed ApcMin mice, at approximately 120 days old showing initial signs of anaemia. Swiss roll preparations of the intestine were used to detect Tns4 by immunohistochemistry. Each animal presented fewer than 12 polyps in the whole intestine, and in 50% of these polyps expression of Tns4 could be detected by immunohistochemistry, whereas normal areas were negative in the small bowel, but positive in the colonic crypts. Tissue obtained from Apc wild-type mice confirmed positive Tns4 expression in colonic crypts, but negative expression in the small bowel. RT-qPCR analysis showed approximately 3-fold upregulation of Tns4 in the polyps compared to adjacent normal tissue. Tns4 overexpression in intestinal polyps of ApcMin mice confirms previous results obtained in cases of human FAP patients and validates the suitability of this mouse model for further investigation of the role of Tns4 in colorectal carcinogenesis.

CD26 Affects Metastatic Potential and Chemosensitivity Across Multiple Colorectal Cancer Molecular Subtypes

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CD26 is a transmembrane glycoprotein expressed on several cell types and has recently been identified to play a role in tumour biology. CD26 expression in colorectal cancer has been correlated to invasion, metastasis and poor clinical outcome. The functional mechanism of CD26 is not yet fully understood, therefore the aim of this study was to investigate the functional role of CD26 in generating the metastatic phenotype in more aggressive consensus molecular subtypes (CMS), specifically CMS3 and CMS4. CD26 was transiently knocked down by siRNA transfection in CRC CMS3 cell lines, HT29 and CL-34, and CMS4 cell line, HCT-116. The effect of CD26 knockdown on cell viability, migration and invasion were assessed, as well as the expression of epithelial mesenchymal transition (EMT) markers. Immunohistochemistry was piloted in a tissue microarray (n=84) to analyse expression of CD26 and associations with clinicopathological characteristics in primary colorectal cancer. Knockdown of CD26 in each cell line lead to a decrease in sensitivity to 5-FU. The migratory and invasive capabilities of each cell line were reduced when CD26 was knocked down ($p<0.05$) alongside changes in EMT-related markers. Expression of CD26 in tumour nuclei had a weak negative correlation to resection margin status and KRAS status ($p=0.05$ and $p=0.049$). The results suggest that CD26 plays a role in tumour progression and metastasis in both. CMS3 and CMS4 CRCs. However, knocking down CD26 may reduce chemosensitivity to 5-FU and hence further studies are necessary to elucidate its functional role in CRC.
This study was supported by a CDF from the Pathology Society.

The Role of CD24/P-Selectin Complex in the Progression of Colorectal Cancer

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Introduction: Colorectal cancer (CRC) is a malignant neoplasm developed from the epithelium of the colon and rectum and metastasise to distant organs. CD24 is highly expressed in CRC cells and it has been shown to have a vital role in inducing cell proliferation, invasion and migration. It also acts as a ligand for P-selectin that is expressed in vascular endothelium.
Methodology: Two CRC cell lines, HCT116 (CD24-ve) and SW620 (CD24+ve cell) were used in this study. HCT116 was transfected with CD24-plasmid and SW620 was transfected with siRNA for CD24. The two cell lines were thereafter treated separately with P-selectin. The effect of P-selectin on CD24 expression and its downstream signalling molecules (Notch1, Cten, FAK, and ILK) was assessed by Western blotting. Functional assays on invasion and migration were also performed. Additionally, adhesion and trans-endothelial migration assays were carried out using endothelial cells (HUVEC).
Results: CD24 expression was increased in SW620 and HCT116-transfected with CD24 plasmid after stimulation with P-selectin. Along with the overexpression of CD24 in the presence of P-selectin, the downstream signalling molecules were also activated. Increased migration and invasion in these cells was observed upon treatment with the P-selectin. In addition, silencing either CD24 or P-selectin in HUVEC decreased the number of CRCs adhesion to HUVEC and similarly reduced their migration through endothelial cells.
Conclusion: P-selectin/CD24 signalling axis is responsible for the aggressiveness in the CRC cells and may facilitate metastasis of CRC cells through the vascular barrier.

CD24 Modulates Angiogenesis in Colorectal Cancer

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Introduction: Colorectal cancer (CRC) is defined as a cancerous growth initiated by unregulated growth of cells or tissues in the internal lining of colon and rectum. The metastatic process requires the establishment of an appropriate local microenvironment, including angiogenesis, to support tumour growth. CD24 gene is highly expressed in CRC cells and has been shown to have a vital role to induce many cancer-like characteristics, such as invasion and migration.
Methodology: Two techniques of tube formation assay were performed to study the effect of CD24 on angiogenesis. Firstly, using conditioned media retrieved from CRC cells (HCT116 and SW620) that had been transfected with overexpressed CD24 plasmid or siRNA knockdown respectively. Secondly, performing an indirect co-culture where the CRC cells were on top of the Transwells insert and the HUVECs were at the bottom well coated with Matrigel. The migration of HUVECs was also studied using Transwells migration assay.
Results: An increase in the endothelial tubular formation was observed by in the presence of CD24 in either HCT116 overexpressing CD24 or SW620 siRNA control. However, in the absence of CD24, a significant inhibition in tube formation was determined. This data was confirmed by using indirect co-culture, which showed similar results. In addition, the number of migrated endothelial cells was increased when the CD24 positive cells were used as a chemoattractant compared with control.
Conclusion: CD24 expression induced endothelial tube formation and migration, which suggests it may have a major role in colorectal cancer progression by stimulating angiogenesis.

TSC22D4 and the Metastatic Phenotype in Colorectal Cancer

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TSC22D4, a gene encoding a leucine zipper protein, has been hypothesized as being a transcription factor. Recent integrated profiling data from the Cancer Genome Atlas Network suggests that TSC22D4 may be a novel gene involved in vascular invasion and metastasis in colorectal cancer. This study investigated functional effects and clinicopathological associations of TSC22D4 in colorectal cancer. TSC22D4 was transiently knocked down with siRNA in CRC cell lines HCT116 and DLD-1. Proliferation, invasion, migration and wound healing assays were performed to determine functional activity. Survival of TSC22D4 knock-down cells treated with 5-FU were investigated by cell viability assay. Western blot was used to determine the expression of TGF-β and VEGF-C proteins. Immunohistochemistry (IHC) was performed on a pilot CRC tissue microarray to assess correlation with clinicopathological variables. TSC22D4 knock-down decreased the rate of migration, more significantly in DLD-1 than HCT116 cells. In DLD-1, knock-down increased TGF-β expression. No significant changes in VEGF-C expression were seen in either cell lines. Knock-down of TSC22D4 has a transient trend of decreased drug sensitivity (24 hours), which becomes abrogated at 72 hours. No significant clinicopathological variables were associated with expression on IHC. TSC22D4 seems to have effects on migration but not on invasion in some CRC cell lines. The lack of clinicopathological and functional correlations, suggests a passenger rather than driver effect and demonstrates the difficulties in translating metadata findings to clinically relevant functional studies.

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Cten is Overexpressed Under Hypoxic Conditions in Metastatic Colorectal Cancer Cell Line

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Introduction: Hypoxia is a condition characterized by low oxygen levels within tissue and is common in malignant tumours. Hypoxia stimulates a multitude of different signaling pathways in cancer cells and can specifically enhance epithelial-mesenchymal transition (EMT) leading to increased cell motility and metastasis. C-terminal tensin-like protein (Cten) is an adaptor protein that has been shown to induce EMT and migration in different cancer types including colorectal cancer (CRC). The goal of our study is to investigate the expression, regulation and role of Cten under hypoxic conditions in CRC.
Methods: The CRC cell lines SW480 and SW620 were incubated under hypoxic (1%O2) and normoxic conditions. Protein was extracted at 24, 48 and 72 hours. Western blot was used to assess Cten protein levels under both conditions. To see if Cten is regulated by HIF-1α, we silenced HIF-1α in SW620 using HIF-1α siRNA. To investigate the impact of Cten on HIF-1α we silenced Cten using Cten siRNA.
Results: Cten protein was upregulated under hypoxic conditions in SW620 but not SW480. Silencing HIF-1α in SW620 seems to reduce the expression of Cten under hypoxic conditions. Silencing Cten in SW620 seems to reduce the expression of HIF-1α under hypoxic conditions.
Conclusion: The upregulation of Cten in CRC under hypoxic conditions is cell line dependent. SW480(primary) and SW620 (metastatic) are derived from the same patients, therefore, it can be possible that Cten is upregulated under hypoxic condition in metastatic tissue only. Cten is regulated by HIF-1α under hypoxic conditions, moreover, the reduction of Cten expression in SW620 result in reduced Hif-1αexpression under hypoxic condition. These data suggest that Cten might be a contributor to hypoxia.

The Value of Modified Davidsons Fixative (MDF) in Lymph Node Retrieval in Gastro-Intestinal (GI) Cancers

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Purpose of study: The identification of lymph node involvement in GI cancer specimens is important for prognosis and treatment. Our on-going study is examining the use of a proven lymph node retrieval solution MDF to establish an accurate and reproducible staging for GI cancers whilst minimally impacting an established diagnostic pathway.
Methods: The histological data was compared in a range of GI cancer specimens with and without MDF treatment. The final impact on diagnosis and the overall effects on integrating MDF into an established diagnostic pathway were examined.
Summary of results: Preliminary results have shown that on average a further 10 lymph nodes per case were found after treatment with MDF (n=16). The majority of these nodes were less than 3mm in size and difficult to see prior to treatment. Furthermore, the majority of these cases would not have had the minimum lymph node yield defined by RCPath guidelines before treatment. The use of MDF has been shown to be of no detriment to immunohistochemistry or molecular tests. The use of MDF has had minimal impact on cost and the established diagnostic pathway.
Conclusions: This preliminary study has shown that MDF has the potential to be a reproducible and accurate method for staging GI cancers whilst minimally impacting an established diagnostic pathway or further prognostic assays. Further work is continuing to establish its benefits in the upstaging in a range of GI cancers.

Challenges and Temporal Trends in the Pathological Diagnosis of Indeterminate Colitis and Inflammatory Bowel Disease Unclassified: A Tertiary Centre Experience

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Arriving at a firm diagnosis of ulcerative colitis (UC) or Crohn's disease (CD) has important treatment implications; however, 5–10% of inflammatory bowel disease (IBD) cases defy subtyping and per RCPath guidelines should be classified as indeterminate colitis (IC) or inflammatory bowel disease unclassified (IBDU) at resection or biopsy, respectively. The aims of this study were to: 1) evaluate histopathologists' adherence to IBD classification guidelines; 2) identify the main challenges in subtyping IBD; 3) identify the broader multi-parameter and temporal diagnostic contexts of IC and IBDU.
Electronic histopathological, endoscopic, radiographic, and clinical records were reviewed retrospectively for patients at a tertiary institution, diagnosed as IC or IBDU between 2008 and 2018 (mean duration of follow-up: ~7 years). There were 16 IC and 110 IBDU cases. IC and IBDU were appropriately labelled in 94% and 96% of cases, respectively. Occasional mislabelling resulted from the use of nonstandard terminology in pathology reports. The main factor that precluded IBD subtyping on colectomy specimens was overlapping histopathological features of UC and CD (88%). The main factors for IBDU diagnosis on biopsy samples were lack of specific histopathological features (43%) and conflicting findings on different modes of investigations (31%). A final diagnosis of IC was preceded by a consistent long-term history of UC in 31% and CD in 13% of cases. The majority of IBDU cases either had an initial diagnosis persisting as IBDU (45%) or were subsequently reclassified (31%) as UC or CD. Strict adherence to standard diagnostic terms is recommended to prevent mislabelling of unclassifiable IBD cases for both clinical and research benefit. Difficulties in subtyping arise even when a multidisciplinary approach is used. Emerging computational and imaging analysis techniques may help process this complex data and improve categorisation of difficult IBD cases.

Increasing Patient Access to MSI Testing with Automated PCR

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Microsatellite Instability (MSI) is now a recognised feature of many common cancers and is the hallmark of Lynch syndrome. The MSI status of many tumours is increasingly sought urgently by oncologists for the MDT to help guide treatment and prognosis. In colorectal cancer (CRC) specifically, the use of MSI testing is well established and NICE guidelines now mandates screening all patients for MSI/Lynch syndrome. Despite this demand and the current national reorganisation of molecular diagnostics, there are difficulties in many centres in accessing testing. The Idylla™ MSI Mutation Assay (research use only, pending CE-IVD approval) offers an easy to use and automated platform that can be placed in any histopathology department and offers rapid, on-demand testing. The platform has shown promising results in other key targets for patients with CRC (BRAF, KRAS, NRAS) and together with new MSI assay the platform can offer complete molecular diagnostics and Lynch screening for patients with CRC. This study aimed to assess the concordance of this novel test with current standard methods. Thirty formalin-fixed, paraffin-embedded CRC tumour cases (15 MSI-High and 15 microsatellite-stable tumours by routine testing) were retrospectively analysed with the Idylla™ MSI Mutation Assay. Twenty seven of the cases had also undergone mismatch repair (MMR) immunohistochemistry (IHC) at the time of initial diagnosis. The concordance of Idylla with routine MSI testing (Promega) was 100% (95% CI 88.65% to 100.00%) and the concordance with IHC was 96.3% (95% CI 81.72% to 99.34%; the concordance of IHC and MSI testing at initial diagnosis was the same). The results show that the Idylla™ MSI Mutation Assay is a potentially accurate alternative to existing methodologies and together with the other assays available on the platform, could easily widen access to routine molecular testing for patients with CRC across the UK.

Infiltrative Tumour Growth Pattern Correlates with Poor Outcome in Esophageal Cancer

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Esophageal cancer (EC) is an aggressive malignancy with a 5-year survival rate of 50% for localized tumours. Accurate prognostic markers which could guide treatment decisions in routine practice are urgently needed. Tumour growth pattern (TGP) has been shown to reflect tumour aggressiveness in a variety of tumours. However, limited data are available on the significance of TGP in EC. We performed a retrospective assessment of TGP in a group of patients with adenocarcinoma or squamous cell carcinoma. We searched for patients who had undergone surgery for EC from 2005 to 2017 at Institut Jules Bordet or Hôpital Erasme. Patients with haematoxylin and eosin stained slides from surgical specimens with a minimum of 10% of residual tumoural area over total tissue area were included. TGP was classified as either pushing (PP) if solid sheets of tumour cells present a well-demarcated tumour-stromal interface, or infiltrative (IP) if cords of tumour cells infiltrate the surrounding stroma in a spray-like pattern. Kaplan-Meier curves, Cox's proportional hazards model and log-rank tests were used with p values considered statistically significant at the bilateral <0.05 level. 101 patients were included. 35 had undergone surgery alone while 66 had received a neoadjuvant treatment (chemoradiotherapy in 23 cases ; chemotherapy in 43 cases). TGP was IP in 61 patients and PP in 40 patients. Patients with tumours with a PP had a significantly better OS (mOS 7,64 years vs 2,42 years) than those with tumours with IP (HR 0,56 [95% CI 0,33-0,93], p=0.03). After adjusting for prognostic variables including histology and disease stage in multivariate analyses, the association between TGP and OS remained unchanged (HR 0,5 [95% CI 0,28-0,9], p=0.02). This study shows that TGP is an independent prognostic factor in EC patients who undergo surgical resection of the primary tumour. Further studies are required to elucidate the molecular mechanisms underlying TGP and to characterize their dynamics.

The Immune Landscape in Esophageal Cancer

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Esophageal Cancer (EC) is an aggressive cancer with an increasing worldwide incidence. With a 5-year survival rate of 50% for localized tumours, immunotherapy research holds the promise of new treatment options. Hence, characterizing the EC immune microenvironment is indispensible. We included 115 patients with adenocarcinoma (ADC) or squamous cell carcinoma (SCC). Surgical specimens were stained for CD3/CD20 or CD4/CD8 and scored for percentage of tumoural surface occupation, either in the intratumoural compartment (IC) or in the migration front (MF), by an experienced pathologist. Tertiary lymphoid structures (TLS) were counted. In case of (quasi-) complete response (pCR) to neoadjuvant treatment, the assessment was performed on the persistent scar. 15 negative proximal margins of gastric cancer were used as control. Mann-Whitney tests were used with p values considered statistically significant at the bilateral <0.05 level. Among 44 SSC, 8 patients received chemotherapy (CT), 19 radiochemotherapy (RCT), while 17 had no preoperative treatment. Among 71 ADC, 38 received CT, 15 RCT and 18 had surgery alone. 78,6% of the pCR cases received RCT, 21,4% received CT. Tumour samples without any preoperative treatment showed stronger infiltration of CD20, CD4 and CD8 than controls (p<0,0001). Treatment naïve SSC tumours were more infiltrated in CD4 than ADC (p=0,02) at the MF and showed stronger decrease in CD4 upon RCT (p<0,0001) than ADC. Remarkably, CD20 was decreased in the RCT group, in ESCC and ADC, in IC and MF (p≤0,0003). TLS were also less in the RCT group compared to patients with surgery alone (ESCC: p=0,004; ADC: p<0,0001). Concomitantly, pCR tumours were less infiltrated in CD20, CD4 and TLS in IC or MF. No association was found between CD8 and different types of histology or treatment received. While current research focuses on CD4 and CD8 cells for immunotherapy development, these preliminary results pinpoint a preponderant role of CD20+ B cells in EC.

A Prognostic Signature of Brown Fat-Associated Proteins in Colorectal Cancer

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Colorectal cancer is a common type of malignancy with a relatively poor outcome and is one of the main contributors to cancer related deaths. Brown fat phenotype/proteins have been implicated in tumour growth and metastasis. Therefore, the aim of this study was to characterise the expression of brown fat-associated proteins cell-death-inducing DNA fragment factor 45-like effector A (CIDEA), elongation of very long fatty acids 3 and 5 (ELOVL3 and 5) and uncoupling protein 1 (UCP1) in colorectal cancer. Monoclonal antibodies to these protein targets were developed with short peptide immunogens which were selected using a range of bioinformatic tools. To select each peptide, the structural and physico-chemical properties of each protein were analysed. The antibodies were used to profile the expression of proteins by immunohistochemistry in a discovery cohort (274 primary colorectal cancers) and in a validation cohort (549 primary colorectal cancers). Unsupervised hierarchical cluster analysis was used to examine the overall relationship of proteins expression with overall survival and based on this identify a protein signature associated with prognosis. Cluster analysis of all proteins identified a cluster that was significantly associated with patient survival in the discovery cohort (HR=1.574, 95%CI=1.037-2.390, χ^2=4.658, p=0.031). Cluster analysis of the validation cohort also showed that the pattern of expression was significantly associated with patient survival (HR=1.691, 95%CI=1.284-2.228, χ^2=14.405, p<0.001). Multi-variate analysis confirmed that the cluster group was prognostically independent of clinically-established prognostic parameters (p=0.03). This study showed that novel targets CIDEA, ELOVL3, ELOVL5 and UCP1 are overexpressed in colorectal cancer. A prognostic signature of these proteins has been identified in colorectal cancer.

Audit of Lymph Node Yield from Colorectal Cancer Resections 2005–2018

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Introduction: The Royal College of Pathologists (RCPath) dataset for reporting of colorectal cancer (CRC) highlights that there is variability between pathologists in the detection of lymph node (LN) involvement. A proportion of cases with low LN yield will be understaged because positive LNs may not have been processed. The dataset advises that the median number of LN examined should be 12 or more. Furthermore, the Scottish CRC Quality Performance Indicator (QPI) states that at least 90% of resection specimens should yield 12 LNs or more. This audit compared LN yield in CRC resections in our centre with the standards set by the RCPath and Scottish QPI.
Methods: A retrospective analysis was performed of anonymised data collected from all CRC resections reported by our centre (a regional cancer centre) from 2005–2018 inclusive.
Results: A total of 3614 cases were analysed. The mean age of patients was 69 and 55% were male. 27% of cases were rectal tumours (median LN yield = 18), 43% in the proximal colon (median LN yield = 19), and 30% in the distal colon (median LN yield = 17). 20% of cases had received neoadjuvant therapy, of which 94% were rectal tumours. Neoadjuvant therapy had little effect on the median LN yield (17 with therapy vs. 18 without). The Dukes stage also had little effect on LN yield (Dukes A = 16, Dukes B = 19, Dukes C = 18). Median LN yield ranged from 14 to 23, with a general trend of increasing LN yield over the period analysed. A median yield of at least 21 LNs was achieved since 2013. A median LN yield of 12 or more has been achieved in at least 90% of cases consistently since 2011.
Conclusions: The data demonstrate that our centre has either met or exceeded the RCPath standard for the past 14 years. Furthermore, the Scottish QPI target has been exceeded consistently since its introduction. The data demonstrate that these targets can be attained consistently in routine practice.

Retroperitoneal Liposarcoma Mimicking a Primary Colonic Tumour: A Case Report

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Retroperitoneal liposarcoma is usually an incidental finding or patients may have non-specific symptoms such as increasing abdominal girth, abdominal and back pain. Some of the very unusual presentation include colonic intussusception with occasional cases reported in literature. We hereby report a 62 year old gentleman who presented with diarrhoea. He was noticed to have a large 15cm intussuscepting polyp in the transverse colon in Computed Tomography. There were several enlarged mesenteric lymph nodes with inflammatory changes in the mesentery. In addition two separate metastatic deposits were also noticed in the psoas muscle. A primary colonic tumour was radiologically considered and the patient underwent a right hemicolectomy. Histological examination revealed a largely ulcerated mesenchymal lesion involving mainly the submucosa and closely associated with the colon. This was composed of malignant pleomorphic spindle cells admixed with a large numbers of inflammatory cells including eosinophils. The spindle cells were positive for S100, Desmin, Vimentin and negative for AE1/AE3, HMB45, Melan A, SOX10, H-Caldesmon and ALK. The case was referred to centralised soft tissue pathological service for further opinion. Expert consultation confirmed well differentiated liposarcoma in the mesenteric fat with a dedifferentiated component mainly seen in the submucosa of the transverse colon that had mimicked a primary colonic intussuscepting polyp. Further retroperitoneal resection revealed extensive well differentiated liposarcoma in the retroperitoneum extending into the right renal hilum and encircling the adrenal gland. This case emphasizes the need for considering dedifferentiated liposarcoma in the differential diagnosis of malignant spindle cell lesions in the gastrointestinal tract and for careful examination of mesentery. This case was unusual as it had presented predominantly as an endoluminal polyp and radiologically also mimicked a primary colonic tumour.

The Two Week Weight: The Increasing Burden of Targets in Histopathology

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Purpose and method of study: Increasing numbers of requests requiring prioritisation are being received by histopathology departments. We have analysed endoscopic specimens received as urgent (U), two week wait (2WW), Bowel Cancer Screening (BCSP) and routine (R) in our department from 2018 and a 'snapshot' week in March 2019 to assess this workload and to consider whether our service is being used appropriately.
Results: Jan–Jun 2018 Total endoscopy requests 7025. 2WW requests 1113 (20.9%). BCSP requests 1096 (15.5%) Jul–Dec 2018 Total endoscopy requests 7398. 2WW requests 1845 (25.4%). BCSP 1013 (13.7%). Week commencing 11 March 2019. Total endoscopic requests 320 (total biopsy sites 357) U=16.2%. 2WW=19.6%, BCSP=22.7%. R=41.5%. Using published guidelines for the appropriate use of histopathology for investigation of the upper and lower GI tract (UGI and LGI), an assessment was made for each specimen (excluding BCSP) in the study week and potentially inappropriate biopsies were recorded.
Potentially inappropriate requests UGI. U 32.0%. 2WW 36.9%. R 29.7%
Potentially inappropriate requests LGI. U 13.3%. 2WW 40.0%. R29.4%
Discussion: The results confirm the high proportion of a large GI workload that requires prioritisation. Prioritisation is disruptive in terms of workflow and impacts on the performance of scientific staff, medical staff and office staff to the detriment of specimens regarded as routine. There are frequent disruptions by specimen trackers and the responses take up additional time for all staff groups. The typing delay for routine specimens was 5 working days during the study week and has sometimes been as high as 10 working days. Additional resources are unlikely to be forthcoming in the current funding climate but the rational of use of histopathology in the investigation of the GI tract could reduce demand. Local discussions with gastroenterologists may be more beneficial than attempts to increase funding.

Nicorandil Induced Enteropathy Clinically Mimicking Neuroendocrine Tumour (NET) of the Small Intestine: A Case Report

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A 66 year old man with a history of high blood pressure, angina, resected diverticular disease and incisional hernia, presented with weight loss, nausea, abdominal cramping and loose stools. A CT scan indicated subacute small bowel obstruction suspicious of a slow growing tumour. Octreotide scan showed local increased uptake suggestive of neuroendocrine tumour (NET). Increased serum Chromogranin A of 8.4nmol/L was also found and the patient underwent small intestinal resection under the cancer pathway. Macroscopic dissection showed a localised stricture with fibrosis, extramural abscess formation and diffuse longitudinal linear punctate ulcers throughout the small bowel. Histology revealed a patchy active chronic enteropathy with multifocal demarcated ulcers with little inflammation. The stricture showed a transmural, undermining ulcer with surrounding fibrosis. No malignancy, microorganisms or granulomas were found. Drug-induced enteropathy due to Nicorandil was concluded following close clinical-pathological correlation. The patient discontinued Nicorandil, with advice to the patient's GP to comprehensively review the patient's current medication regimen. He has remained well, with no further angina attacks. Nicorandil is a commonly used drug in the symptomatic treatment of angina. Rare, but known, complications of Nicorandil use include gastrointestinal ulceration, which may progress to perforation/abscess or fistulation. This case highlights that gastrointestinal side effects of Nicorandil can rarely clinically impose as a tumour. Increased awareness of potential Nicorandil induced enteropathy may help to recognise early lesions that may still respond to drug cessation. Nicorandil induced ulceration/perforation enters the differential diagnosis when encountering gastrointestinal ulceration of unknown cause at pathological examination.

The Utilisation of CD31/AE 1/3 Dual Staining to Identify Venous Invasion in Colorectal Cancer

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Objective: To examine whether the use of dual staining (DS) for CD31\AE 1\3 will improve the detection of venous invasion (VI) in colorectal Cancer (CRC).
Background: Extramural venous invasion is a well-established independent prognostic indicator in CRC .Assessing VI is a requirement for TNM8. DS can be used to improve the detection of VI
Methods: A single- centre, retrospective review including the detection of VI using DS in 72 blocks from the first 18 CRC specimens, Stage I to III, reported in2019. Talbot's definition of VI was used subdivided into intramural (IM) and extramural (EM). The dual staining was carried out on a Ventana Bench Mark XT using monoclonal CD31 and AE1/AE3 antibodies. Ventana Ultra view detection kits where used alongside Ventana DAB and Ventana universal AP red chromogen systems.
Results: VI (IM and EM combined) was detected in 66.6% (12/18) on Haematoxylin and Eosin (H&E) preparations. 55.5% (10/18) was EMVI and 11.1% (2/18) IMVI. The use of DS for CD31/AE1/3 improved the detection rate of VI (IM and EM combined) to 88.8% (16/18).IMVI was identified in 38.8% (7/18) and EMVI in 50% (9/18).
Conclusion: The result of the current study introduces a novel approach with increased VI detection rate (88.8%) as compared to the previously published data using elastic staining which offers 56% detection rate. IMVI has been proven in recent meta-analysis to also be of prognostic significance. Standard use of DS amongst non-specialist gastrointestinal pathologists may enhance VI detection rate including IMVI.

Audit to Assess the Impact of the Royal College of Pathologists Colorectal Cancer Dataset in a District General Hospital

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Purpose: Colorectal cancer remains the third most common cancer in the UK and surgical resection the mainstay of treatment. To improve outcomes and standardize pathological assessment the Royal College of Pathologists (RCP) has outlined a colorectal cancer dataset. This audit aimed to assess the impact of this in a district general hospital.
Methods: Pathology reports for colorectal cancer resection specimens generated between two separate time periods (Jan 2014 – Dec 2014 and Apr 2017 – Apr 2018) were quality assessed against RCP dataset guidelines. These two time periods were then compared using the Fisher exact test.
Results: 229 pathology reports were generated between 2013–2014 and 99 for 2017–2018. In both groups the reporting of tumour site, tumour type, the extent of local invasion and lymph node status was 100%. Venous invasion, tumour differentiation and involvement of the longitudinal resection margin were reported in >90% in both groups. In the 2013–2014 group the reporting of certain parameters was below standard: maximum tumour diameter reported in 85.2% of cases, tumour distance to nearest longitudinal margin in 74.7%, tumour perforation in 19.7%, circumferential resection margin involvement in 74.2% and distant metastasis in 77.7%. Conversely these parameters were reported in >90% of the 2017-2018 group cases. When compared this was significant (p=0.0001). Additionally the median lymph node harvest increased from 13 in the earlier group to 17 in 2017–2018. Furthermore the proportion of cases with a lymph node harvest of >12 has increased significantly (p=0.0001).
Conclusions: Regular audit has been shown to improve patient outcomes in many specialties. This audit, undertaken in a district general hospital, has shown that the introduction of RCP guidelines has not only improved the quality of pathology reporting over time but also routine clinical practice.

Comparison of Pathological and Radiological Reporting of Extramural Venous Invasion in Rectal Cancer

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Purpose: Extramural venous invasion (EMVI) is a poor prognostic indicator in colorectal cancer and an important factor in determining management during MDT discussion. EMVI detection is a core data item in the colorectal dataset. A detection rate of 25% is recommended. Evidence suggests that MRI may be more sensitive and provide an earlier opportunity to detect EMVI in rectal cancer compared to histological reporting. Royal College of Radiologists (RCR) guidelines advocate including EMVI in the MRI report. This audit aims to assess and compare the pathological and radiological reporting of EMVI in rectal cancer over time at a district general hospital.
Method: Pathology and MRI reports following rectal cancer resection generated between two separate time periods (Jan 2014 – Dec 2014 and Apr 2017 – Apr 2018) were quality assessed against RCP and RCR guidelines. These two groups were then compared using the Fisher exact test.
Results: During 2013–2014 there were 42 rectal cancer resections with both pathology and MRI reporting available and there were 26 for 2017–2018. Over 2013–2014 EMVI was included in 90.4% of pathology reports. Of those 18.4% were EMVI positive. In the 2017–2018 group EMVI was reported in 98% of pathology reports. Of these EMVI was detected in 24%. The increase in pathological reporting of EMVI between these time periods was proportionally significant (p=0.02). Comparatively EMVI was included in only 4.8% of the 2013–2014 MRI reports and 7.7% of the 2017–2018 reports. There was no significant improvement in MRI reporting rates of EMVI over time (p=0.63).
Conclusions: Clinical audit can help to improve patient outcomes and clinical practice. This audit has shown that pathology reporting of EMVI has improved over time whilst radiological reporting of EMVI has not and is overall poor. Regular inter-specialty audit and discussion should be performed to help improve communication between specialties, highlighting areas for improvement.

Audit on Comparison of Oesophageal Brush Cytology with Biopsy and Endoscopy in the Detection of Oesophageal Candida

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Purpose of the study: Endoscopic sampling when oesophageal candidiasis is suspected includes brush cytology and biopsy. Although studies have compared the relative yield of brush cytology to biopsy, it is not clear what is best practice for the diagnosis of oesophageal candidiasis. The aim of this audit is to evaluate the yield, sensitivity, and specificity of brush cytology vs. biopsy in the diagnosis of oesophageal candidiasis.
Methods: This is a retrospective review of 141 oesophageal brushings from 01/2014 to 02/2017 at a university hospital. Cytology results were compared to the final histology, where available, and endoscopy to assess the overall sensitivity and specificity.
Summary of results: Of the 141 cases, 68 had all three tests completed. Of the 68 cases, Candida was seen on cytology in 50 (74%) and on both biopsy and cytology in 6 (8.8%) cases. 63 of the 68 cases (92.6%) were identified to suspect Candida on endoscopy. 48 of the 63 cases (76.2%) were found to be positive on cytology. Two of 5 cases not suspected to have Candida on endoscopy had positive cytology. Endoscopy had a sensitivity of 0.96 (CI = 0.85-0.99) and a specificity of 0.16 (CI = 0.04-0.42), cytology had a sensitivity of 1 (CI = 0.91-1) and a specificity of 1 (CI = 0.78-1), biopsy had a sensitivity of 0.12 (CI = 0.05-0.25) and a specificity of 1 (CI = 0.78-1) for the detection of oesophageal Candida.
Conclusions: The sensitivity of brush cytology is significantly higher than biopsy and better than endoscopy for presence of oesophageal Candida. Endoscopy with brush cytology diagnosis appears to be an ideal method for diagnosing oesophageal Candida.

Primary Malignant Melanoma of Oesophagus: A Rare Case Report

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Introduction: Primary Malignant Melanoma of the Oesophagus (PMMO) is a rare and aggressive malignancy, accounting for only 0.1 to 0.2 % of all oesophageal malignancies. Mean age of diagnosis is 60.5 years with male preponderance. We report PMMO in a young female with past medical history of stage V Wilms tumour at the age of 2.5 years with pulmonary metastasis treated with right nephrectomy and chemo-radiotherapy. Rare reports of malignant melanoma have been described following treatment for Wilms tumour.
Case report: A 34-year female presented with 6 months history of intermittent dysphagia, more persistent for last 6 weeks. Endoscopy revealed a 58 mm polypoid mid-oesophageal tumour and biopsy showed malignant melanoma. The tumour was staged as T3N2M0 on PET-CT scan and EUS. Two-stage oesophago-gastrectomy was performed.
Histology and follow up: Histology confirmed an ulcerated malignant melanoma with Breslow thickness of 6.2mm, mitotic count of 30/mm2 and lymph node metastasis. Junctional activity and in-situ melanoma were noted in the adjacent oesophageal squamous mucosa. Molecular testing showed no evidence of BRAF or KIT gene mutation. The tumour was staged as pT3N2M1 (oesophageal carcinoma), pT4bN3M1c (skin melanoma) and pT3N1M1 (upper aero-digestive tract melanoma). Based on morphology and immunohistochemical phenotype, and in the absence of a primary melanoma elsewhere, a diagnosis of PMMO was rendered. Despite palliative treatment with Nivolumab, the patient developed widespread metastatic disease and sadly died within 3-months of diagnosis.
Discussion: PMMO is a highly aggressive tumour with a poor outcome despite radical surgery and chemo-radiotherapy. Due to its rarity, our understanding of tumour biology is limited and there is current lack of consensus on treatment and staging guidelines. Recent molecular studies have shown different mutations in PMMO than seen in melanoma at other sites and this may be helpful in targeted immunotherapy for better survival.

Colon Biopsies: Too Many Pots!

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Maidstone Hospital receives specimens from three hospitals (A, B, C). A discrepancy was anecdotally appreciated between these sites in regards to the numbers of colonic biopsies received, specifically the number of specimens for "normal" colonoscopies. An investigative audit was undertaken to corroborate this observation and assess whether this practice was congruous with current guidelines.
Method: A retrospective Telepath search was performed from 1st Oct 2017 – 31st Dec 2017 using T codes for all lower GI specimens. Resection specimens, biopsies for dysplasia and miscoded cases were excluded. Data was collected for: hospital site, number of pots per case, number of biopsies per pot per case, clinical indication and final diagnosis. The data was analysed using an Excel spread sheet.
Results: A total of 1208 cases were identified for the 3 month period. This translated to 2885 pots and 6784 biopsies in total. The number of cases per site per month were roughly equivalent, however, site A submitted consistently more pots and biopsies per month than sites B and C. Site A had, on average, 4 pots per case compared to sites B and C with 2 pots per case. This difference was demonstrated further when analysed in terms of frequency of pot numbers per case in terms of "low pot numbers" (1–3 pots) and "high pot numbers" (6–9 pots). The distribution was skewed towards low pot numbers for sites B and C whilst site A had a more even distribution causing a flattened profile of the frequency curve. The high pot number cases were sub-analysed. Site A had a total of 95 cases with high pot number (30% of total cases). Sites B and C respectively had a total of 34 and 28 cases (9 and 5% of total cases).The histological diagnostic distribution however, was equivalent across all sites despite biopsy practices varying.
Conclusion: A substantiated discrepancy exists between site A when compared to B and C. This provides scope for rationalisation and standardisation of biopsy practice.