Dublin Pathology 2015
Supplement

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Department of Pathology,
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Venue
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Companion Sessions
Association of Clinical Electron Microscopists
Renal EQA
UK NEQAS ICC & ISH

Supplement Contents

KEY

Presenter = Ⓟ

Abstracts marked ◆ have not been subjected to peer review

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

◆ Typing and Terminology – Tips on Key Diagnoses in Ovary, Fallopian Tube and Endometrium

WG McCluggage

Belfast Health and Social Care Trust, Belfast, UK

The pathologist is playing a pivotal role in the identification of patients with familial gynaecological cancer syndromes even when there is no personal or family history of neoplasia. This is because the tumour types which occur in the various syndromes are generally fairly constant and predictable. Familial cancer syndromes in which neoplasms may occur in the female genital tract include BRCA1/2, Lynch syndrome (hereditary non-polyposis colorectal cancer syndrome), Peutz-Jeghers syndrome, DICER1 syndrome and hereditary leiomyomatosis and renal cell carcinoma syndrome. Given the close association between genotype and phenotype, the pathologist has a key role in raising the possibility of an underlying cancer syndrome and accurate diagnosis is essential to this end. In the uterine corpus, carcinomas associated with Lynch syndrome tend to be endometrioid rather than non-endometrioid in type and a proportion of these neoplasms are difficult to categorise. It is probable that only high grade serous carcinomas (which in most cases arise from the fallopian tube fimbria rather than the ovary) are associated with BRCA1/ BRCA2 germline mutations. Rarely, other ovarian tumours occur in patients with germline BRCA1/ 2 mutation but these may be coincidental or may represent misclassified high grade serous carcinoma. A scenario can be envisaged whereby all patients with ovarian/ tubal high grade serous carcinoma undergo BRCA testing and all patients with ovarian endometrioid or clear cell carcinoma undergo testing for Lynch syndrome. As such, pathologists need to provide accurate diagnosis. The role of WT1 and p53 in distinguishing between high grade serous carcinomas and endometrioid, low grade serous or clear cell carcinoma in problematic cases is stressed, as is the fact that mixed carcinomas in the ovary are very uncommon.

◆ Identifying Hereditary Predisposition in Patients Presenting with Gynaecological Malignancies – An Update on Non-BRCA Non-Lynch Hereditary Gynaecological Cancer Syndromes

BA Clarke

Toronto General Hospital, Toronto, Canada

Large cohort studies and next-generation sequencing studies have significantly advanced our understanding of hereditary gynaecological cancer syndromes, highlighting robust genotype-phenotype correlations. Tumour subtype designation by pathologists conveys genetic (both somatic and germline), prognostic and therapeutic information. Criteria for cancer genetic consultation referral include tumor type in combination with genealogy based criteria and sometimes tumour type alone (histology based criteria). Pathologists are playing an increasingly pivotal role in identifying hereditary predisposition in cancer patients. It behoves us to be aware of such associations and to advocate for genetic consultation referral when reporting appropriate cases. Furthermore, such genetic alteration may be of predictive value, influencing therapy selection and may also provide meaningful quality assurance of histologic assessment. Regarding gynaecological malignancies, the phenotype of DICER 1 syndrome is now known to include ovarian sex cord-stromal tumours, uterine cervix embryonal rhabdomyosarcoma and primitive neuroectodermal tumor in addition to pleuropulmonary blastoma, cystic nephroma, and multinodular goitre. Recent identification of SMARCA4 somatic and germline mutation in small cell carcinoma of the ovary, hypercalcemic type, has facilitated accurate diagnosis and genetic counselling as well as prompting reconsideration of tumour nosology. Other important syndromes in which histologic tumour assessment can be critical are Hereditary leiomyomatosis/ renal cell carcinoma syndrome and Peutz-Jeghers syndrome with its associated sex cord tumor with annular tubules, Sertoli cell tumour and adenoma malignum/ gastric type endocervical adenocarcinoma. Despite the vicarious nature of the patient-pathologist relationship, pathologists are crucial in the recognition and management of hereditary cancer syndromes

◆ Identifying Lynch Syndrome in Patients with Gynaecological Malignancies: Implementation of Reflex Testing and its Implications

BA Clarke

Toronto General Hospital, Toronto, Canada

Patients with Lynch syndrome (LS) are at significantly elevated lifetime risk for several cancers including ovarian and endometrial (EC) carcinoma. A gynaecological malignancy will be the sentinel cancer in the majority of women with LS. Identification of LS accrues advantages to the patient, kin and the health care system. MMR is also a prognostic (TCGA) and possibly predictive marker (immunotherapy.) Predicated on poor performance of genealogy and morphology schemas, reflex testing of gynaecologic cancer specimens with mismatch repair immunohistochemistry (MMR-IHC) has been proposed. Based on a prospective study showing MMR-IHC to be the superior screening strategy to identify LS in women with EC, we have adopted reflex screening of all patients with EC using MMR-IHC. Adoption of reflex testing using MMR-IHC has resolved some abiding contentions and raised new requirements to be addressed. It is recognised that MMR-IHC loss for MSH2/ MSH6 may result from somatic mutation, dismissing debate that MMR-IHC is akin to germline testing. Laboratories need to engage in quality assurance programs of performance and interpretation of MMR-IHC. Audits are required to ensure that tumours from all eligible patients are indeed tested with MMR-IHC with appropriate subsequent referral to genetic counsellors. Pathologists need to be cognizant of pitfalls in IHC staining and interpretation. Reporting must be standardized. Education is required to ensure the clinicians understand reports and respond appropriately. Finally the "circle of care" has to integrate the multidisciplinary team: pathologists, clinicians, family doctors, genetic counsellors and medical geneticists with sharing of information. Since the laboratory investigation of an individual patient may occur at different sites, is iterative and may involve both germline and somatic testing of MMR genes, access to genetic information by pathologists is imperative for quality assurance and comprehensive patient management.

◆ A Mainstreamed Oncogenetic Pathway Delivers Fast, Affordable Routine BRCA Testing for Ovarian Cancer (OC) Patients

Ⓟ AJ George[1]; D Riddell[2]; V Cloke[2]; M Gore[1]; S Bannerjee[1]; H Hanson[1]; N Rahman[2]

[1]Royal Marsden Hospital, London, UK; [2]Institute of Cancer Research, London, UK

The development of targeted agents such as PARP-I has made germline BRCA mutation status vital for optimal OC patient (pt) management. Over 15% of OC pt carry a BRCA mutation, exceeding the NICE testing threshold, yet few are tested. We implemented a mainstreamed 'Oncogenetic' pathway to make BRCA testing standard for OC pt. Initially, pt with serous/endometroid OC <65 yrs at diagnosis were offered BRCA gene testing in their Oncology appt by clinicians who had completed a 30 minute online training package; this later extended to all non-mucinous OC pt. Test results were returned by Genetics to the pt; mutation carriers were reviewed in Genetics to arrange other screening and family testing. Patients could contact Genetics at any time, or be referred at the discretion of the clinicician. We tested 119 pt in the first 6 months; 85% had serous tumours. Pt were tested in first line (27%), relapse (38%) or follow-up (35%); mean turnaround was 25 days. 20/119 had a mutation — 8 BRCA1 and 12 BRCA2, 12 had no family history of BC or OC. OC treatment immediately changed in 45% of carriers. All pt were sent a questionnaire to assess the protocol. 100% were happy to have had testing, all within oncology. All understood implications for them and their family. All clinicians agreed BRCA testing was important, and felt confident in offering it. The Oncogenetic model of testing allows flexible, patient-centred, equitable, high throughput gene testing with considerable time and cost savings compared to model of referral to Genetics. It identified a number of patients with mutations who would not have been identified using standard referral criteria, and has resulted in alterations in clinical management. It is now the standard pathway for BRCA testing in OC pts at Royal Marsden and has been adopted by multiple other centres.

◆ Intraoperative Diagnosis

Ⓟ CM Corbishley

St George's Healthcare NHS Trust, London, UK

The use of frozen section diagnosis has decreased in recent years but it still has its' uses. The only indication for an intraoperative consultation is when the outcome will make a difference to the current operative procedure. The pathologist and surgeon need to be aware of the pitfalls and limitations of the technique and the pathologist needs to be both decisive and safe. The talk will specifically cover the use of frozen sections in the field of cancer diagnosis including risk assessment and how to approach the frozen section cases in the final FRCPath Practical examination.

◆ Macroscopic Examination

Ⓟ M Sheehan

Galway University Hospital, Galway, Ireland

This talk will focus on how to approach the macroscopic pathology component of the FRCPath part 2 practical exam. It will also briefly look at how to approach the OSPE stations in this exam section. The aim is to translate from the exam experience back to day to day bench experience with which the exam candidate is very familiar. The application of this day to day knowledge in a focused approach will ensure the candidate answers all the required questions competently. For the macroscopic cases, focus will be on overall description, block sample and compliance with minimum datasets. For the OSPEs, focus will be on a template of key issues/components which must be included in the addressing and discussing the OSPE scenario. The talk will conclude looking at key overall challenges of exam time management, answer construction, clear thinking processes and clear communication of the candidates knowledge for maximum achievement in the exam.

◆ Soft Tissue Cutaneous Tumours

Ⓟ MB Leader

Beaumont, Dublin, Ireland

This presentation will discuss the approach to the diagnosis of cutaneous soft tissue sarcomas. It will discuss spindle cell tumours, myxoid tumours, epithelioid tumours and fatty tumours. Particular emphasis will be given to the differential diagnoses of these lesions and to practical advice on achieving a diagnosis. The commonest cutaneous spindle cell sarcoma of atypical fibro Xanthema will be discussed and the clinical behaviour and morphology will be contrasted with pleomorphic dermal sarcoma, leiomyosarcoma, spindle cell carcinoma and spindle cell melanoma. Three particularly difficult myxoid tumours will be highlighted including myxoinflammatory fibroblastic sarcoma, myxofibrosarcoma and fibromyxoid sarcoma. The differential of myxoma and nodular fasciitis and malignant fibroma histiocytoma will be compared and contrasted. Newer markers (immunohistochemical and molecular) will be discussed. The talk will briefly touch on lipoma like liposarcoma subtle sarcomas such as synovial sarcoma, epitheloid sarcoma and clear cell sarcoma will be demonstrated. Finally, the pitfall of pseudosarcomas with hints of how to avoid these pitfalls will be discussed.

◆ Primary Bone Tumours: Guiding Treatment Using Histology and Molecular Genetic Alterations

Ⓟ AM Flanagan

UCL Cancer Institute, London, UK

Reaching a diagnosis of a primary bone tumour is facilitated by using genetic alterations which are characteristic of such lesions. However, the pathologist's interpretation of the histology in the context of the radiology is still required for choosing the relevant tests to help arrive at a diagnosis. Benign osteoclast-rich tumours include giant cell tumour of bone, chondroblastoma and aneurysmal bone cyst and these must be distinguished from an osteoclast-rich osteosarcoma and clear cell chondrosarcoma. Over 90% of giant cell tumour of bone and chondroblastoma harbour a recurrent H3F3A and H3F3B substitution: approximately 70% of aneurysmal bone cyst harbours a USP6 rearrangement: this alteration is also found in 90% nodular fasciitis. However, ~1% of high grade osteosarcoma also harbours a H3F3 substitution: copy number change can distinguish these osteosarcomas from giant cell tumour of bone and chondroblastoma. Approximately 50% of conventional central chondrosarcoma, the most common adult primary bone tumour, harbours an isocitrate dehydrogenase (IDH) substitution (94% IDH1, 6% IDH2) but this does not help in determining tumour grade. CDKN2A and or TP53 alterations only occur in high grade tumours. An IDH alteration is particularly useful in distinguishing dedifferentiation chondrosarcoma from an osteosarcoma and is clinically relevant as the treatment is different. A GRM1 rearrangement in chondromyxoid fibroma is helpful in distinguishing this tumour from chondrosarcoma. Low grade fibrous tumours in bone include low grade osteosarcoma (central and parosteal) and fibrous dysplasia and the treatment for these differ: MDM2 amplification is found in the former and GNAS alterations in the latter: they are mutually exclusive. Development of multiplexing technology will make the delivery of these tests more efficient but the interpretation of the molecular data in the context of the histology, and clinical information will remain central to good clinical practice.

◆ Toxicology: Interpretation for the Pathologist

Ⓟ SP Elliott

ROAR Forensics, Malvern, UK

Toxicology concerns the study of the effects, nature and detection of drugs and poisons and their dose. The involvement of drugs or poisons may not always be immediately obvious to Pathologists. Even in instances where there is significant evidence to suggest the ingestion of drugs has occurred, the exact drug (or more usually drugs) may not be known. Furthermore, even if a drug has been detected, the interpretation of its presence or amount can depend on many factors. Not least the type, nature and actions of the drugs themselves. It is also important to be aware of the wider issues that impact on toxicology, including; appropriate sample collection, analysis, drug stability and possible endogenous production of compounds. There are a number of considerations for toxicology interpretation namely; there is no fatal range for all drugs - some overlap with therapeutic range, drug tolerance, pharmacogenomics, drug combinations, drug stability, post-mortem production and importantly, post-mortem redistribution (which also means in life data does not equate to post-mortem data). The Pathologist should be aware of these factors in order to consider the potential role of drugs in a death in conjunction with the findings at autopsy and the case circumstances. Whilst appropriate interpretation is a constant challenge for all drugs, something of significant pertinence to modern toxicological casework is the rise of so-called "legal highs". This has become a particular issue within the last decade and it is a major challenge to keep up with this either analytically (i.e. actually detecting such drugs) or interpretatively (i.e. what is the significance of the presence or concentration of a drug when little is known about it?). Overall, there are many factors that have to be considered to enable valid and appropriate interpretation of toxicology findings and Pathologists should bear in mind that rigid toxicology "ranges" do not exist.

◆ Diagnosis of Infection, Sepsis and SIRS at Autopsy

Ⓟ SB Lucas

St Thomas' Hospital, London, UK

Problems arise in interpretation of systemic aspects of sepsis (systemic inflammatory response syndrome — SIRS) and its large differential diagnosis. The end result of severe sepsis always involves a haemophagocytic syndrome (HPS) = macrophage activation syndrome = haemophagocytic lymphohistiocytosis. The causes of HPS are genetic (inherited) and reactive (acquired) forms: Inherited genetic defects of perforin gene on chr 9q21, the MUNC13-4 gene on chr 17q25, or mutations in syntaxin 11 gene on chr 6q24. Infections: viruses such as EBV and influenza; HHV8 infection in HIV disease; numerous bacteria (eg group A Strep and Staphylococcus — toxic shock syndromes), M.tuberculosis; leishmaniasis. Autoimmmune disorders: eg adult onset Still's disease and vasculitis. Lymphomas: B-cell, T-cell and Hodgkin lymphomas can all present with HPS, even when the tumour volume is low (or occult); Common to all are the following generic features: haemophagocytosis of blood cells: these are optimally seen using macrophage immmunohistochemistry (IHC: CD68, PGM-1) where the macrophages are increased in number and size, containing engulfed cells in addition to their nucleus; seen in marrow, liver, spleen and nodes; upregulation of intercellular adhesion molecule-1 (visualised with CD54 IHC), seen best in lung vessels; acute lung injury; the spleen may be small with white pulp atrophy, or enlarged in other presentations including HHV8-associated multicentric Castleman disease HPS; the kidney may show disseminated intravascular coagulation, as part of a coagulopathy.

◆ Minimally Invasive Coroner's Autopsy – The Present and (Near) Future

Ⓟ ISD Roberts

John Radcliffe Hospital, Oxford, UK

The role of post mortem imaging is changing. In the past it was used only as a supplement to dissection in forensic practice, to reveal details of fractures and identify foreign bodies. In the last 20 years, cross-sectional imaging has been increasingly used as a replacement to standard coroner's autopsy, for those cases in which the family object to invasive examination. Early services lacked both evidence base and governance. Recent research has defined the strengths and weaknesses of post mortem imaging, optimised imaging protocols and led to technical improvements, most notably the development angiographic techniques. It is now possible to accurately identify those cases for which imaging alone is sufficient to diagnose the cause of death. CT scan is generally superior to MRI for the investigation of sudden adult deaths; using CT with angiography, three quarters can be diagnosed without the need for an invasive procedure. Furthermore, imaging is superior to dissection in identifying certain types of injuries and thus facilitates the recognition of unnatural deaths that might have been missed using traditional autopsy. In the UK, there are currently 6 centres that provide post mortem imaging services, performing several hundred cases annually. There are plans to open a further 16 CT units nationally, dedicated for post mortem work, and it is possible that post mortem CT will soon be used in the majority of coronial cases. Pathologists must become familiar with the application of imaging techniques if they are to be involved in the decision making process, and in particular the selection of cases for which an invasive autopsy is required.

◆ From Research Bench to Patient – Impact and Future of Molecular Diagnostic Developments on Lung Cancer Pathology Practice

Ⓟ S Lantuejoul

CHU A Michallon and J Fourier University, Grenoble, France

Lung cancer is the second most frequent type of cancer but by far the most frequent cause of cancer-related deaths. Up to 60% of lung adenocarcinoma and up to 50-80% of squamous cell carcinoma have known oncogenic driver mutations or translocations, most frequently leading to permanent tyrosine kinase activations targeted by specific drugs (tyrosine kinase inhibitors TKI); the pathologists are now not only expected to provide precise diagnoses whatever the size of the specimen but are also involved in the management of those specimens and the identification of new targeted genetic or molecular abnormalities; among them, EGFR mutations occur in 10% of adenocarcinoma and are detected by most platforms; with the development of NGS technologies, large panels of genes, including KRAS, BRAF, PIK3CA, ERBB2, PTEN, NRAS, STK11, DDR2, MET, FGFR3, FGFR1, will be investigated in all lung cancers; ALK and ROS1 rearrangements are detected in nearly 5% of adenocarcinoma by FISH/immunohistochemistry by most platforms, but pathologists will be soon asked to detect new translocations (RET, NTRK1,..) and new protein overexpressions involved in secondary TKI resistance (C-MET hyperexpression/ amplifications, Axl expression,..). In addition, pathologists will likely participate to the selection of good responders to anti PD1/PD-L1 immunotherapies using PD-L1 immunohistochemistry.

◆ Role of Cytopathology in the 2015 Molecular Era

Ⓟ J McCarthy

Cork University Hospital, Cork, Ireland

Cytopathologists and Histopathologists are most challenged when the sample is small, the cells are few and the differential diagnosis is wide. As diagnostic techniques become less invasive, tissue on which to diagnosis and sub-classify thoracic malignancy becomes less abundant and we as pathologists are being asked to do more with less. EBUS can sample a variety of pathologies in addition to primary lung carcinoma including lymphoma and melanoma that may also require molecular testing. The "Molecular era" brings with it therefore the challenge to offer an accurate diagnosis in addition to the reservation of sufficient material on which to perform molecular tests, affording the opportunity to target mutations specific to individual cancers. In our institution; cytology samples account for 46% of samples tested for EGFR mutatation in Lung cancer and yield 43% of the mutations detected in our tested cohort. There is no statistically significant difference between cytology and histology samples in terms of adequacy of material for EGFR testing. At the same time, the concordance rate for sub typing of Lung cancer between histology and cytology samples is 100%. Achieving this balance requires a combination of curtailed immunostains, co-ordinated handling of separately submitted histology and cytology samples, attempting molecular tests on samples not previously considered worth testing, and for the future, looking at techniques that may purify malignant cells to enhance a molecular signal above background noise. The Pathologist needs to be ready to embrace this era, preferably to be on site for targeting lesions with fine needle aspiration, and ideally situated in close proximity to the molecular testing laboratory to maximise the potential of each individual sample. This may be particularly challenging in institutions without dedicated Cytopathology or in those receiving material from external sites.

◆ Challenges of the New WHO Classification in the Diagnosis of Adenocarcinoma

Ⓟ KM Kerr

Aberdeen University Medical School, Aberdeen, UK

The new WHO classification of adenocarcinoma (ADCA) follows directly from the IASLC/ATS/ERS publication of 2011 in establishing a more clinically relevant, multidisciplinary approach to lung ADCA. The classification includes pre-invasive disease and establishes a category of in-situ (AIS) and minimally invasive (MIA) ADCA, whilst removing the diagnosis of bronchioloalveolar carcinoma. Several variants of ADCA are removed, some remain, sometimes under a new name, and resected tumours are now principally classified according to their predominant histological patterns. An important addition is guidance and nomenclature for ADCA diagnosis in small biopsy and cytology samples. Finally, efforts have been made to make the classification relevant to the radiological diagnosis of this disease as well as the molecular characterisation of tumours for the purposes of selecting patients for targeted therapy.

Whilst resolving several problematic issues with the 2004 classification, several challenges either remain or have emerged. The diagnosis of pre-invasive disease (atypical adenomatous hyperplasia or AIS) is difficult since lesions are uncommon and criteria quite subjective. The identification of invasion is frequently problematic. Accurate identification of a predominant pattern in invasive tumours, and especially the enumeration of other patterns present, is sometimes quite challenging and a pragmatic approach is needed. Immunohistochemistry (IHC) has emerged as a crucial tool is diagnosis. Not only is it now part of the definition of solid pattern ADCA in resected cases, it is pivotal in accurate small sample diagnosis. Molecular characterisation of ADCA is now a routine standard of care. With this comes the need for technically consistent IHC, careful tissue handling to ensure IHC and molecular testing are possible, and complex workflows designed to ensure the complete diagnosis required for patient management.

◆ Staging / Classification of Thymic Epithelial Tumours

Ⓟ A Marx

University Medical Centre Mannheim Universty of Heidelberg, Mannheim, Germany

Staging of thymomas has not changed significantly since Masaoka and Koga proposed their stage I-IVb scheme. Although not validated for thymic carcinomas (TCs) it has ever since been applied to them, despite the very different propensity of thymic epithelial tumours (TETs) for lymphogenous and haematogenous metastasis (that is frequent only in TCs). Nevertheless, the International Association for the Study of Lung Cancer (IASLC) and the International Thymic Malignancy Interest Group (ITMIG) have jointly proposed an evidence-based TNM staging system that is founded on the analysis of >8.000 clinically annotated cases, is applicable to thymomas and TCs and awaits approval by UICC/AJCC. This new staging system will be presented. The 4th edition of the WHO Classification of thoracic tumours was published in March 2015. It puts emphasis on an interdisciplinary perspective on thymic tumours by depicting state-of-the-art CT or PET/CT images and incorporating cytology. Epidemiological and prognostic data were updated by mining the world-wide, retrospective database of the ITMIG that compiles over 6000 case of thymic tumors. The refinement of morphological criteria mainly addresses the "borderlands" between type A and AB thymomas, between type B1-3 thymomas and between type B3 thymoma and thymic squamous cell carcinoma, aiming to improve reproducibility and clinical relevance. For the first time immunohistochemical criteria are suggested for the diagmosis of difficult-to-classify cases and a new "atypical type A thymoma variant" is introduced to reflect the realization that histological features (like comedo-type necrosis) may be associated with the rare occurrence of advanced tumor stage, including metastasis, in type A (and AB) thymomas. The lecture will detail the differences between the 3rd and 4th edition of the WHO classification of thymic epithelial tumors.

◆ Approach to the Diagnosis of Rare and Unusual Lung Tumours

Ⓟ AG Nicholson

Royal Brompton and Harefield NHS Foundation Trust, London, UK

Any primary tumour arising in the lung apart from carcinomas and carcinoids falls under the umbrella term of a "rare lung tumour". This therefore includes other epithelial tumours, soft tissue tumours, lymphomas and even teratomas and melanomas. These have recently been updated in the WHO 2015 classification, with the last ten years seeing greater understanding of the histogenesis of certain rare neoplasms, such as sclerosing haemangioma being definitively identified as a low-grade epithelial neoplasm. New tumours with specific genetic abnormalities have also been identified such as pulmonary myxoid sarcoma with EWSR1-CREB1 translocation, and entities such as lymphangioleiomyomatosis (LAM) and Langerhans cell histiocytosis are now also classified as neoplasms. It is useful therefore to have a uniform approach to a potential neoplasm that does not look like a common entity. My approach to these tumours is to use the WHO 2015 classification as a template, then to correlate this with its morphological (e.g clear cell, spindle cell etc.) and immunohistochemical profile to see whether the proposed diagnosis is consistent with these data. I then review the case again in relation whether the presentation of the tumour is appropriate for my proposed diagnosis (benign/low-grade versus malignant cytology, tumour location, solid/cystic, local/diffuse) and finally reviewing it once more in the context of whether tumour might be a secondary process or even a reactive process. In this way, even the rarest tumour should be able to be correctly classified.

◆ The Management of B3 Lesions with Emphasis on Lobular Neoplasia

Ⓟ AM Shaaban

Queen Elizabeth Hospital Birmingham, Birmingham, UK

B3 lesions are a heterogeneous group of different pathological diagnoses including those with and without atypia. They comprise flat epithelial atypia (FEA); atypical intraductal proliferation (AIDP); in-situ lobular neoplasia (ISLN); papillomas, radial scars, fibroepithelial lesions and other. Approximately 20-25% of all B3 lesions are upgraded to cancer on further tissue. examination and the upgrade rate is higher in the presence of atypia across all B3 lesions. The management of B3 lesions has traditionally been via diagnostic surgical excision biopsy. However, it is becoming increasingly recognised that at least some of the B3 subtypes may only be associated with a low risk of malignancy and therefore surgical excision for all may represent over-treatment. The introduction of vacuum assisted biopsy (VAB) offers an alternative option to surgery in the management of such B3 lesions. VAB can yield up to 3.6g of tissue providing a robust method for thoroughly sampling B3 lesions diagnosed on needle core. Therefore, patients could avoid unnecessary surgery if there is confidence that the lesion has adequately been sampled by second line VAB and the diagnosis has not been upgraded to malignancy. VAB is also advantageous if it confirms a malignant diagnosis as the patient can progress straight to therapeutic surgery. Currently, there is lack of consistency in the management of those lesions across screening centres in the UK. Guidelines are therefore being developed. Current and future management plans will be discussed in detail with emphasis on ISLN.

◆ Update from The Sloane Project

Ⓟ JS Thomas

Western General Hospital, Edinburgh, UK

The Sloane Project has closed to new cases of DCIS and data consolidation is complete for the 12,500 cases entered between 2004 and 2012 with over 90% of patients having a complete four-specialty (radiology, surgery, pathology and radiotherapy) data set. Over the period of case acquisition the Steering Group published eight peer-reviewed papers on radiological diagnosis and pathological correlation, the surgical management of DCIS particularly in relation to the axilla and variation in practice around the UK in the assessment of oestrogen receptors, radiological lesion size estimation, pathological evaluation of specimens, mastectomy rates, particularly for small lesions, and treatment with radiotherapy. In the second phase of the Project we are continuing to acquire patients with ADH and lobular neoplasia but are shifting our emphasis away from audit *per se* towards the biology of the disease. With a median of five years follow up to date we have data on over 750 recurrences/events in our patient cohort and are looking at the dataset for features associated with these events. We also have ethical approval and funding to support an examination of tissue from Sloane Project cases to look for biological markers associated with outcome measures. We are also collaborating closely with the organisers of the LORIS Trial evaluating the role of non-surgical intervention for low risk DCIS.

◆ Genetics of Cardiomyopathies

Ⓟ ER Behr

St George's University of London, London, UK

The presentation will describe the current state of genetic knowledge in relation to cardiomyopathy. It will primarily focus upon the sub-phenotypes of hypertrophic, arrhythmogenic and dilated cardiomyopathies. Current issues with 'genetic noise' will be addressed and the role for genetic testing on a clinical basis will be further discussed. Genetic variation in cardiomyopathy genes associated with unexplained sudden death and the Brugada syndrome will also be explored.

◆ Clinico-Pathological Correlations on Vasculitis

Ⓟ A Fabre; Ⓟ E Molloy

St Vincent's University Hospital, Dublin, Ireland

This lecture provide four examples on different patterns of small and large vessels vasculitis as well as their underlying etiologic factors, to assess the clinic-pathological correlation. All cases discussed with have clinical and radiological data and the discussion will emphasis on tissue sampling, histological patterns and differential diagnoses, both by microscopy and clinically, including autoimmunue associations and drug related changes. This will provide an opportunity to bring the clinical aspects of vasculitis into the histopathological evaluation of vascular changes in light of improved imaging available to clinicians.

◆ Sudden Cardiac Death in Athletes

Ⓟ S Sharma

St George's University of London, London, UK

The benefits of regular exercise on the cardiovascular system are established; therefore the sudden death of an athlete from a cardiac cause sends shockwaves through the lay community. Over 80% of all non-traumatic deaths in athletes are attributable to abnormalities of the heart. The prevalence of sudden cardiac death (SCD) in athletes is generally in the range of 1 in 50,000. Over 90% of SCDs affect males. Athletes of African or Afro-Caribbean origin are at greater risk compared with Caucasians. Deaths are most common in dynamic sports of a start-stop nature such as soccer and basketball. Hypertrophic cardiomyopathy is the commonest cause of SCD worldwide, whereas arrhythmogenic right ventricular cardiomyopathy is the leading cause in Italian athletes. There is emerging data from US athletes and soldiers and from a tertiary UK center that these diseases may account for up to 30% of all deaths. Pre-participation screening to identify athletes at risk of SCD is recommended by learned scientific communities. ECG screening is effective at detecting high risk ion channel diseases and congenital accessory pathways. ECG screening is also helpful for raising suspicion of the cardiomyopathies. A 25 year old prospective ECG screening programme in Italian athletes revealed a 90% reduction in the prevalence of SCD in athletes from 3.6/100,000 to 0.4/100,000. ECG screening is associated with high incidence of false positive results and is ineffective for detecting coronary artery disease. Furthermore deaths from acquired conditions such as myocarditis, heat injury and electrolyte disturbances cannot be predicted, therefore the availability of personnel trained in cardiopulmonary resuscitation and automated external defibrillators (AEDs) at sporting arenas is prudent. Data from high schools with AEDs and mass participation events shows that early CPR and use of AEDs has a survival to discharge of up to 65%.

◆ Update of the Classification of Renal Carcinomas

Ⓟ S Fleming

University of Dundee, Dundee, UK

The classification of renal cell carcinoma has been based on the underlying genetic changes since pioneering work in the early 1990s. As new genetic alterations are identified and corresponding morphological criteria developed the classification is regularly updated through the work of the ISUP and WHO consensus meetings and publications. The latest version of the WHO Classification (Version 4) is due to be published shortly. It recognises several lesions described since the 2004 classification and further emerging entities which require further characterisation. It is now recognised that there exists a family of renal tumours exhibiting translocations involving the MiT family of transcription factors, most commonly TFE3 on Xp11 but others involving TFE B on chromosome 6 are now well described. Tubulocystic carcinomas have a typical morphology and behaviour, previously classified as low grade collecting duct carcinoma they have now been re-classified. Mutation of the fumarate hydratase gene encoding a member of the mitochondrial electron transport chainis seen in the hereditary leiomyomatosis and renal cll carcinoma syndrome (HLRCC). RCC formation is accompanied by loss of the wild type allele and gives rise to a morphologically recognisable tumour type. A second mitochondrial gene, succinate dehydrogenase B is also seen in a morphologically distinct form of RCC. These several new tumour types will be presented.

◆ Update in Penile Pathology: The new Dataset and Diagnostic Pitfalls

Ⓟ CM Corbishley

St George's Healthcare NHS Trust, London, UK

Penile tumours are rare with only 600 cases of cancer and precancer diagnosed in the UK per year. The last 12 years have seen a major change in the way that these tumours are dealt with surgically with increasing subspecialisation and the formation of cancer supranetworks which are well developed in England and Wales. Over the last 14 years at St George's in London we have seen over 1100 new cases of penile, scrotal and distal urethral cancer and have pioneered the use of sentinel node evaluation and penile preserving surgery. 2015 sees the publication of the new WHO blue book on Urological cancers and the new Royal College of Pathologists Cancer dataset for reporting of Penile and Distal Urethral cancers. Much has changed since the previous 2006 dataset edition. The terminology of precancerous lesions is now encompassed by the term PeIN (Penile Intraepithelial Neoplasia) and the entity differentiated PeIN has been described. New subtypes of penile cancers have been identified and the association of some tumours with HPV subtypes, particularly type 16, is well established. Sentinel node evaluation has been shown to be reliable and prevents unnecessary debilitating groin dissections. Penile preserving surgical techniques, such as glansectomy and glans resurfacing with plastic reconstruction, are used wherever possible to avoid formal penectomy and give good cancer control. The major pitfalls in penile cancer pathology include difficulties in diagnosing PeIN subtypes and accurate invasive tumour grading and subtyping. The anatomy of the penis is complex and accurate staging requres a detailed knowledge of anatomy. Some of the well differentiated variants, verrucous carcinomas and pseudohyperplastic carcinomas, are difficult to diagnose especially in small biopsies. As with other rare tumours, consistency of reporting is improved with experience and the opportunity to report significant numbers of cases within a specialist multidisciplinary team.

◆ Updates in Bladder Cancer Including the RCPath Dataset, Subtypes of Bladder Cancer and Molecular Developments

Ⓟ JH Shanks

The Christie NHS Foundation Trust, Manchester, UK

One controversy in preparing the RCPath "Dataset for tumours of the urinary collecting system [2nd edition])", April 2013 was which WHO grading scheme (1973 or 2004) to use for urothelial carcinoma. Since there is a split within grade 2 (1973) between low grade & high grade, use of both schemes in parallel was recommended. This has the advantage of better indicating where a particular patient lies in the grading continuum & minimises the consequences of 'grading error' for cases close to the threshold between low & high grade in the 2004 scheme, (a critical distinction for management if 2004 WHO grading is used in isolation). The 2015 NICE guidance on bladder cancer (http://www.nice.org.uk/guidance/ng2) incorporates risk stratification tables for Ta/T1 bladder cancer utilizing this parallel WHO grading recommendation in a multiparameter formula that also considers tumour size, pT classification and the presence/absence of CIS or aggressive subtype(s). Amongst the more aggressive bladder cancer subtypes is invasive micropapillary urothelial carcinoma and the nested variant. A bladder origin can be difficult to recognise at metastatic sites, especially for micropapillary and discohesive/plasmacytoid subtypes. Uroplakin II has recently become available, is more sensitive than uroplakin III & may assist within a panel. Spindle cell lesions of the bladder are often difficult & loss of cytokeratin expression is common in sarcomatoid carcinoma. There is potential to mistake inflammatory myofibroblastic tumour for a malignant tumour. Two molecular pathways for bladder cancer are recognised. *FGFR3* at 4p16 is the most frequently mutated oncogene in bladder cancer & is prevalent in low grade papillary tumours. *p53* loss of function mutations and loss of RB1 are prevalent in CIS. No prognostic molecular test is currently validated for clinical use in bladder cancer, though some (e.g. FISH) are in use as an adjunct in diagnosis.

◆ 'New' Observations About Old Entities in Testicular Pathology

Ⓟ TM Ulbright

Indiana University School of Medicine, Indianapolis, USA

Some dogmas in testicular pathology do not hold up to scrutiny. The belief that all pure, postpubertal teratomas are malignant is invalid. Within this group there is a small subset that is benign; these may be divided into dermoid and non-dermoid types that, however, share features that distinguish them from the usual teratoma of adults. These include absence of: atypia, regressive parenchymal changes, intratubular germ cell neoplasia, and i(12p). Also they usually show organoid arrangements, and prominence of ciliated epithelium, squamous cysts and smooth muscle. Patients do not need further intervention beyond excision sufficient to establish the diagnosis. Cases often interpreted as isolated testicular polyarteritis nodosa because of the presence of fibrinoid vascular necrosis are mostly attributable to chronic, intermittent torsion. They usually present as pain-associated, palpable or ultrasound-detected masses, with the "mass" corresponding to infarct and/or hemorrhage. There are associated chronic vascular changes, with frequent marked intimal hyperplasia of arteries, mural fibrosis of veins, dilated venules and arteriolar hyalinization, consistent with torsion-induced venous outflow obstruction and secondary arterial hypertension. These patients do not develop systemic vasculitis on follow-up. Many "sarcomas" in patients with germ cell tumours, especially after chemotherapy, are more correctly regarded as sarcomatoid yolk sac tumours. They are reactive for cytokeratin and glypican 3. They often show characteristic features: nodular growth, myxoid and fibrous stroma, spindled and epithelioid cells, abrupt changes in cellularity, and tumor "ringlets." Most are high grade and aggressive. Most regressed germ cell tumours can be recognized through a combination of findings, although the only diagnostic ones are a scar with coarse intratubular calcifications or with intratubular germ cell neoplasia.

◆ Dying for a Drink – The Problem with Alchohol (and Some Solutions)

Ⓟ FE Murray

Royal College of Physicians of Ireland, Dublin, Ireland

Alcohol consumption has doubled in Ireland and the UK in the last 60 years. The causes of this increase include increased affordability of alcohol and its widespread availability. As a consequence, the health harms associated with alcohol have dramatically increased. Binge drinking and alcohol consumption among by women have risen especially dramatically. For example, the mortality from cirrhosis has doubled in the last 20 years in both men and women. In response to this, the medical profession on both islands have led informal and later formal campaigns to encourage policy change regarding alcohol at a national level. In Ireland, this was driven by RCPI. The involvement of the medical profession has had a powerful influence, as doctors do not have a conflict of interest in this matter, in contrast to the alcohol industry. The policy changes advocated include particularly Minimum Unit Pricing (MUP), which has been shown to be effective in reducing alcohol consumption, alcohol-related admission to hospital and crime in Canada. Modelling of the data suggests it would have similar benefits in UK and Ireland. This is regarded as the single most important first step. Other steps which will help include actions around alcohol labelling, availability and breaking the link between alcohol and sports and leisure promotion. Turning off the tap of cheap alcohol will hopefully soon reduce alcohol health harms in UK and Ireland

◆ Barrett's Oesophagus: An Evolving Challenge for the Gastroenterologist

Ⓟ DOT O'Toole

St James's Hospital & St Vincent's University Hospital & Trinity College Dublin, Dublin, Ireland

Cost-effective surveillance programmes for Barrett's oesophagus (BO) needs to be focused at risk groups. It is not only a question of identifying more individuals with BO (initial screening) but screening and subsequent surveillance has to identify at-risk individuals with BO who can benefit most from surveillance or therapy. Advances in endoscopic imaging (high resolution endoscopy ([HRE] with dye-based chromoendoscopy, electronic chromoendoscopy, and autofluorescence,...) certainly prove beneficial in better detecting dysplasia within known BO and can guide sampling and subsequent therapy. Dysplasia can be patchy and easily missed during routine biopsy sampling of BO and adequate training with high resolution instruments is needed to increase detection rates. Once dysplasia is detected, endoscopic ablation is recommended. Until recently, the standard treatment for HGD was oesophagectomy but endoscopic resection and ablation techniques are now available to eradicate dysplasia and mucosal adenocarcinomas. Resecting visible lesions (using endoscopic mucosal resection [EMR] or endoscopic submucosal dissection techniques) allows full pathological T staging. When invasive cancer is eliminated at multidisciplinary review (i.e., purely mucosal neoplasia confirmed with a nodal risk <2%), further endotherapy to ablate residual metaplasia in BO can be performed using radiofrequency ablation (RAF). In a tertiary centre use of staging EMR is frequently necessary (>60%) in patients referred for endotherapy and expert endoscopy is required to ensure safe and complete oncological resection. Conversely pT1 submucosal cancers detected following EMR are confidently triaged for oesophagectomy. Combination of EMR and RFA in expert groups exceeds >95% for eradication of neoplasia and metaplasia. Adverse events are quite low (stricture form healing, 4%; self-limited haemorrhage, very rare perforations ...) and recurrence of BO is also low (<10% at 5 years). Careful follow-up endoscopies is necessary at 3 to 6 months initially; intervals theerafter probably yearly.

◆ The Pathologist's Role in the Diagnosis and Management of Neoplasia in Barrett's Oesophagus

Ⓟ C Muldoon

St. James's Hospital, Dublin, Ireland

The last decade has seen a revolution in the management of neoplasia in Barrett's oesophagus. More detailed biopsy protocols, along with the advent of sophisticated local resection and ablation techniques, have radically altered the management of this expanding cohort of patients. These new treatment modalities, coupled with a massive increase in the numbers of cases of Barrett's being diagnosed, have significantly altered the demands placed upon pathologists involved in this area. These changes have presented pathologists with an opportunity to challenge our existing practices, to improve the reproducibility of our analysis and to increase the clinical relevance of the way in which we report neoplasia in this setting, where the pathologist plays a critical role in the multidisciplinary management approach. This talk aims to outline a practical approach to the handling of these specimens and to provide clear guidelines as to how to report them in the most clinically useful way.

◆ Modern Management in IBD

Ⓟ MWR Vieth[1]; H Neumann[2]

[1]Klinikum Bayreuth, Pathology, Bayreuth, Germany; [2]University Hospital Erlangen, Medical Clinic I, Erlangen, Germany

Ulcerative colitis and Crohn's make a distinct histological picture. Around 80% of an IBD diagnosis is the clinical information and about 20% derives from histology, only. For routine purposes it is recommendable in case of a first manifestation of an IBD to make a diagnosis such as: "picture of ulcerative colitis or picture of crohn's disease" and to recommend a follow-up endoscopy with biopsies not prior to 8 weeks after the first endoscopy and than confirm the diagnosis later to exclude mimickers of IBD. In Crohn's disease it is helpful to take biopsies from the upper GI-tract to get further hints of Crohn's disease. In case of neoplasia, the guidelines leave some room for local endoscopic treatment of low grade dysplasia whereas high grade dysplasia is still seen as an indication for operation since there is a high probability of detecting a carcinoma in the operation specimen afterwards. Operation means on normal complete proctocolectomy. This has been indivdually questioned in the last time. There are exceptions for discussing the indication of an operation in IBD: cases with numerous pseudopolyps that cannot be searched for neoplasia, low grade lesions that cannot be completely removed, multiple neoplastic lesions and unresponsiveness to medical treatment.Operation and endoscopic specimen in IBD need a subtile search for neoplastic lesions. A microscope with reverse light may help to identify suspicous lesions and may help to decide where exactely to cut a specimen. In conclusion a tight cooperation between clinical and histopathological partners is recommended to reach a high standard for patient care. Second opinions may help to achieve and fuel the own learning process esp. in an institution with a lower number of IBD patients during the year.

◆ The Pathology of Colorectal Cancer Screening

Ⓟ MB Loughrey

Royal Victoria Hospital, Belfast, UK

Colorectal or bowel cancer screening (BCS) is commonplace and organised national screening programmes have been developed in many countries, most notably in western Europe. Traditionally, faecal occult blood (FOB) detection has been the screening method of choice, those testing positive being selected for subsequent colonoscopy, but this is changing, with alternative or additional screening tests gaining favour. Most of the problems in BCS pathology practice are particularly related to FOB-based screening programmes, as these are enriched for large, bleeding sigmoid adenomas, in comparison to programmes utilising endoscopy as the primary screening modality. Experience within the closely related UK BCS programmes to date has yielded several recurring problems: the diagnosis of stage pT1 or 'polyp' cancers, in particular distinguishing common epithelial misplacement from 'true' invasion; the management of stage pT1 cancers, in relation to indications for surgical intervention after such a diagnosis; and the minimum criteria for a biopsy diagnosis of colorectal adenocarcinoma. These issues will be discussed with illustrative examples, along with the somewhat more mundane but highly important practical issue of measuring various parameters related to BCS pathology. The importance of quality assurance measures to ensure high standards within BCS pathology is emphasised.

◆ Predicting Lymph Node Metastatic Disease in pT1 Polyp Cancers

Ⓟ ID Nagtegaal

Radboud UMC, Nijmegen, Netherlands

With the introduction of colorectal cancer screening in various countries of the EU there is a sharp increase in the incidence of early colorectal cancer. A significant part of these early tumours presents in a pedunculated polyp. In most cases, these carcinomas are already completely removed by polypectomy. Classic risk factors that suggest a high risk for lymph node metastases include Haggitt level 4, positive resection margins, poor differentiation and lymphatic or vascular invasion. However, the evidence is rather thin. Most pT1 studies are performed on sessile polyps. Risk factors are more firmly established and include differentiation grade, lymphatic invasion, Kikuchi level sm3 or the presence of budding. However, for a clinical useful decision model, we will need an integrated approach, and both specificity and sensitivity of the various factors should be taken into account. Radical surgery seems overtreatment for a large number of polyp cancers.

◆ Colorectal Cancer: Updated Royal College of Pathologists' Guidelines

Ⓟ P Quirke

Leeds University, Leeds, UK

Minimum datasets have changed cancer reporting. This talk will explain the decision making processes behind the latest datasets both for cancer reporting and bowel cancer screening. It will also look at where we may be going in the future for staging and molecular reporting.

◆ Infective Pathology in the Intestines

Ⓟ MR Novelli

UCL, London, UK

The intestines play host to a broad spectrum of infective organisms ranging from viruses, through bacteria , fungi and unicellular parasites to worms. The spectrum of infections seen varies with geographical location, due to socioeconomic factors and due to changes in human behaviour. Immunocompromisation due to infections (in particular HIV), malignancy (especially haematological tumours) and the use of immunosuppressive drugs also has an important role in determining the infections commonly seen in the GI tract. The typical pathological features of intestinal infections will be discussed together with suggestions on how to optimise the diagnosis of such pathologies.

◆ Unusual Colitides

Ⓟ P Demetter

Erasme University Hospital, Brussels, Belgium

Pathologists are confronted with different types of colitis, most commonly infectious colitis and inflammatory bowel disease (IBD) followed by microscopic colitis and ischaemic colitis. Several other forms of colitis, however, exist and might be underrecognised; these diseases include segmental colitis associated with diverticulosis, diversion colitis, eosinophilic colitis and Behcet's colitis. Clinical presentations of these rare types of colitis vary, and laboratory data are often non-specific; mucosal biopsy is essential in establishing the diagnosis. Segmental colitis associated with diverticulosis (SCAD) is mainly characterised by the involvement of the sigmoid colon with sparing of the rectum and proximal colon. SCAD often mimics IBD at endoscopic and histological examination; since SCAD has a self-limited course that resolves without further recurrence or need for treatment, the implications of an inaccurate diagnosis are obvious. Diversion colitis is a non-specific colonic inflammation following surgical diversion of the faecal stream. Is is characterised by a chronic lymphoplasmacytic infiltrate, and the existence of lymphoid follicular hyperplasia is considered to be a hallmark feature. The development of diversion colitis is attributed to a lack of short chain fatty acids. Eosinophilic colitis is etiologically obscure and can be associated with involvement of other sections of the gastrointestinal tract. An infiltrate of eosinophilic granulocytes is found to varying degrees in all wall layers. A history of food intolerance or allergy is present in most of the patients, and peripheral eosinophilia is present in 80% of cases. Gastrointestinal involvement has been reported in up to 25% of patients with Behcet's disease. In cases with ileocolonic involvement, it is often difficult to distinguish Behcet's disease from other inflammatory bowel diseases. The diagnosis, therefore, often depends on clinical manifestations and intestinal ulcerative lesions.

◆ How to Write a Paper and Get it Published

Ⓟ CS Herrington[1]; Ⓟ DM Berney[2]

[1]University of Edinburgh, Edinburgh, UK; [2]Barts Health NHS Trust, London, UK

Scientific papers have a predetermined structure, and writing in this way requires practice. Most Journals accept only a small fraction of submitted papers and it is important that any paper has something specific to say; and says it in a clear and concise way that can be understood by editors, reviewers and readers, all of whom play a role in assessment of its contribution. Editors look for novelty and significance in the context of the aims and scope of their Journal; and scientific rigour, which expert reviewers help them to assess. Writing a paper and having it assessed by a Journal is an iterative process. During the writing phase, the scientific rigour of the argument can be refined; and following submission and peer review, reviewers and editors often make constructive comments that help to improve it still further. The peer review process therefore acts not only as a quality filter but also as a mechanism for quality improvement. Writing papers and submitting them for publication is therefore generally a positive experience, particularly if one remembers that the process is iterative and (inevitably) not all papers will be accepted for publication by the first Journal that they are sent to.

◆ Large-Scale Routine Diagnostics Using Whole-Slide Imaging in Sweden – the Linköping Experience

Ⓟ C Lundström

CMIV, Linköping University, Linköping, Sweden

This presentation will describe the large-scale routine usage of WSI at Linköping University Hospital, Sweden. Since 2011 all histology slides are scanned, amounting to more than half a million slides to date. To a significant extent the digital images are used for primary review. The initial implementation led to several of the benefits foreseen with digital pathology, but it could also be concluded that further development was needed to unlock the full potential, in particular within the IT solutions. Therefore, a consortium led by CMIV, Linköping University was formed in 2012 to create innovations for a new generation of digital pathology. This triple helix consortium also includes industry and more than half of Sweden's health care providers, an engagement that reflects the dominating view in Swedish pathology that large-scale adoption of WSI practice is possible and desirable. This talk covers the experiences made during the initial digitization, including laboratory process adjustments, and the later additions to the digital pathology toolbox accomplished by the ongoing innovation project. Apart from obvious targets such as the pathologists' workstation, the developments also touch upon other areas including grossing and enterprise image management.

◆ Digital Pathology – Are We There Yet?

Ⓟ SM Hewitt

National Cancer Institute, Bethesda, Maryland, USA

The implementation of whole slide imaging for diagnostic histopathology is far more complex than connecting an instrument to a server, and placing a computer on a pathologist's desk. The technology is additive to the histology workflow, with additional cost beyond the current practice of review with a microscope. The adoption of Digital Pathology for histomorphologic diagnosis requires the restructuring of the workflow, additional technology advances beyond the imaging instrument, and development of new tools to assist the pathologist. The end goal is to improve pathologist's productivity and provide additional diagnostic information. Digital Pathology, to succeed must become a value-added proposition. The adoption of Digital Pathology requires: 1)Improvements in scanner performance as measured by defined quality metrics. 2)Advancement in server and networks to distribute images to the desktop efficiently. 3)Software to facilitate review and diagnosis, beyond presenting only and image of the slide. Evolution of the current technologies is required to provide an economic impetus for widespread adoption and use of Digital Pathology in the diagnostic setting.

◆ Benign versus Malignant Melanocytic Lesions: Lesional Symmetry, Maturation and Ascent

Ⓟ WJ Mooi

VU Medical Centre, Amsterdam, Netherlands

To a significant extent, the distinction between melanocytic naevi and malignant melanomas is based on tissue architecture. Amongst the best known architectural features pointing to malignancy are absence of lesional symmetry and maturation, and presence of melanocyte ascent. However, each of these three features has significant pitfalls. As a rule, naevi are 'roughly symmetrical' and melanomas are not, but there are asymmetrical naevi (traumatized naevi, most larger congenital naevi; some combined naevi; some large acral and genital naevi) and symmetrical melanomas (including many small melanomas, especially small nodular melanomas; some spitzoid melanomas). In addition, it is not always clear whether a lesion should be considered 'roughly symmetrical' or not. I suspect that not uncommonly, a diagnosis is reached first, and the verdict regarding symmetry is adjusted according to that diagnosis. Similar caveats relate to absence of maturation as an indicator of malignancy. It is seen in blue naevi and all its variants; deep penetrating naevi; some BAP1 naevi. Melanomas not uncommonly feature smaller cells in their deeper parts, or there may be an underlying naevus remnant with smaller cells. Naevi with ascent include many Spitz naevi; Reed naevi; some naevi in early infancy; traumatized naevi; naevi of acral skin. Over-interpretation of ascent may result from inexperience with Melan-A and some other immune stains. Melanomas devoid of ascending melanoma cells comprise a wide variety of subtypes including, desmoplastic melanomas and, vexingly, some spitzoid melanomas. These architectural features must, therefore, be evaluated in the context of all other findings, and with a 'splitter's' mind set, taking into account the individual characteristics of the specific naevus and melanoma variants that are of relevance to the case under study.

◆ Melanoma Variants

Ⓟ JE Calonje

St John's Institute of Dermatology, London, UK

Most melanomas are fairly easy to diagnose on histological grounds. However, melanoma is a tumour that can histologically mimic almost any other tumour including epithelial and mesenchymal neoplasms. Pathologists need to familiarize with the wide histological appearances of melanoma to avoid serious misdiagnoses. Of crucial importance is the knowledge that a number of melanomas can closely mimic benign naevi. Some variants of melanoma represent distinctive clinicopathological entities and these include desmoplastic melanoma, "malignant" blue naevus, pigment synthethizing melanoma, naevoid melanoma, spitzoid melanoma and epidermotropic metastatic melanoma. Tumoral melanosis refers to complete regression of a melanoma, a diagnosis that it is often missed because of the absence of tumour cells within the regressed area. A small percentage of melanomas display focal or extensive histological changes that closely mimic other neoplasms and often a combination of histological features with immunohistochemistry is necessary to arrive to the correct diagnosis. Microscopic variants of melanoma include adenoid (pseudoglandular), angiotropic and angiomatoid, signet ring cell, balloon cell, clear cell, rhabdoid and follicular (with exclusive involvement of hair follicles). Some melanomas display heterologous differentiation also known as transdifferentiation. The latter should not be confused with the so-called collision tumour in which a melanoma co-exists with a neoplasm of different lineage. A wide variety of heterologous differentiation has been described in melanoma including osteosarcomatous and chondrosarcomatous (mainly seen in acral melanomas), meiomysarcomatous, rhabdomyosarcomatous, neuroendocrine, ganglioneuromatous and even epithelial. Except for desmoplastic and pigment synthetizing melanoma, all other variants of the tumour have the same behaviour as ordinary melanomas.

◆ Spitzoid Tumours

Ⓟ T Brenn

WGH, Edinburgh, UK

Melanocytic tumours with Spitzoid features represent one of the most challenging and controversial areas in Dermatopathology. What is currently known as Spitz naevus was initially reported as "juvenile melanoma" by Sophie Spitz on 1948. She recognized the relatively indolent but somewhat unpredictable behaviour of these distinctive melanocytic lesions that are particularly common in young children. Over the years, the histological spectrum of these tumours was expanded, and it has become clear that classical Spitz naevi follow an entirely indolent disease course. The prognosis of tumours with atypical histological features remains somewhat unpredictable. This presentation will give an overview of the morphological spectrum of Spitzoid melanocytic tumours, their behaviour and recent advances of their molecular characteristics.

◆ A Research Career in Pathology

Ⓟ NP West

Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, UK

The number of pathologists actively engaging in research has risen again over recent years following the introduction of a defined academic training pathway and the support of bodies like the Pathological Society. A research career is exceptionally rewarding and offers the chance to undertake potentially ground breaking research alongside clinical practice. An interest in pathology research can begin as an undergraduate and a number of opportunities now exist to allow students to explore a research career and embed themselves within academic pathology groups at an early stage. Most medical schools offer special study modules where students can apply to do a piece of research in an area of interest. Several students also opt to undertake research placements during the summer holidays. This can be taken further with a full year out from a medical degree to intercalate a BSc/ MRes, or even undertake a three year Doctoral degree. Following university, academic foundation placements allow for a four month period of research and lead into specialist training pathways where further experience can be gained as an academic clinical fellow (ACF) with up to 25% of time spent in research. This is usually followed by three years out of clinical training to complete a higher degree and leads into a clinical lectureship (CL) where 50% of time is spent undertaking research at an increasingly independent level. ACF and CL opportunities are advertised across the UK in centres of academic excellence. On completion of clinical training, a senior lectureship allows continuation of academic activities at a senior level alongside working as a consultant. A research career is stimulating and varied and offers the chance to undertake research in a world class environment. You get the opportunity to travel widely and experience pathology practice across the world as well as present your work to the scientific community. It is a career path highly recommended by the speaker!

◆ Bioinformatics Analysis to Identify Processed Pseudogenes in Breast Cancer

Ⓟ EJV Ew; POG O' Gaora

University College Dublin, Dublin, Ireland

Pseudogenes are mutated copies of genes which are generally considered non-functional. However, new evidence shows that pseudogenes play a role in tumour biology. Processed pseudogenes (PPGs) arise from reverse transcription of mRNA and transposition into the genome. In this pilot study, breast cancer genomic data were subjected to a bioinformatics workflow to detect potential PPGs. Whole exome sequencing data from 5 normal breast tissue samples and 5 breast cancer tumour samples were downloaded from The Cancer Genome Atlas repository. Reads were aligned to the human genome using the splice-aware aligner STAR. Uniquely aligned reads which aligned across exon-exon junctions (splitreads) were identified for further processing. The splitread counts in tumour samples were compared to those in normal tissue to identify significant differences in potential PPGs. Hierarchical clustering analysis showed clear differentiation of normal and tumour samples. From 23,230 genes in the annotated genome, 2,597 were found to harbour aligned splitreads. Using negative binomial distribution testing implemented in DESeq, 30 genes were found to show statistically significant elevations or depressions in the tumour samples ($p<0.05$). This study identifies 30 genes as potentially important PPGs in breast cancer genomes. Nevertheless, further lab work needs to be performed to confirm the presence of pseudogenes. Furthermore, increased numbers of samples will provide greater statistical power for detection of PPGs. Pseudogene signature profiles may eventually be correlated to tumour subtypes and disease outcome. In conclusion, this study is the first step to understanding breast cancer pseudogene biology.
This study was supported by the Pathological Society Undergraduate Elective Grant.

◆ Student Perception of Optimisation Compared with the Evidence of Optimisation in Paediatric Radiography Case Studies

Ⓟ J Doyle; K Matthews; A McGee

University College Dublin, Dublin, Ireland

Optimisation is a core tenet in radiography and involves the radiographer ensuring that images of diagnostic quality are produced with minimum radiation dose burden to patient and staff [1,2]. In paediatric practice this is particularly important due to the more radiosensitive nature of the child [1]. In alignment with the ISRRT 2013 World Radiography Day theme 'Radiographers Optimise Dose', radiography students in an institution submitted clinical case study coursework that focused on paediatric radiation dose optimisation. The purpose of the current study was to analyse these case studies as examples of prevailing radiographic practice and to compare students' perception of optimisation with the evidence within each case. The evidence of optimisation was established through independent and objective image analysis along with thematic analysis of the case commentaries. The case study evidence demonstrated that optimised techniques were generally well implemented. The exception was collimation, which was sub-optimal in 84% (n=31) of the examinations, and on average irradiating an area 27% larger than necessary. Students were generally able to correctly identify techniques as optimal or not. However, when appraising exposure, positioning and collimation, between 9% and 33% of students were inaccurate in their assessment of what is optimal. Overall the study reflects positively on current Irish paediatric radiography with regard to dose optimisation, although more accurate collimation needs to be practised. Similarly student perceptions show good understanding of optimal techniques, although appreciation of exposure, positioning and collimation errors could be improved.

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◆ Design of a Paediatric Immobilisation Device for use in Radiographic Examinations

Ⓟ JPM Murtagh[1]; MD Davis[2]; EOC O'Cearbhaill[3]; JG Grehan[2]

[1]UCD School of Medicine, Dublin, Ireland; [2]UCD School of Health Sciences, Dublin, Ireland; [3]UCD School of Engineering, Dublin, Ireland

The aim of this project was to design and prototype immobilisation devices for children who are unable to independently maintain upright sitting posture during radiographic investigations. While current market devices exist, they are seldom used by radiographers - particularly in Europe as their methods of restraint have been deemed 'culturally unacceptable' with some claiming that they are in violation of the human rights of the child [1]. The design challenge was to create devices that were functional (fit for purpose [2], radio-lucent, compliant with infection control and easy to use) while minimising discomfort and intimidation. A search of the literature, prior art, patent landscape and current market devices was performed in order to identify product requirements. TRIZ methodologies - a problem solving, analysis and forecasting tool derived from the study of patterns of invention in the global patent literature were used to identify the physical contradictions underlying the design challenge and generate potential solutions. Eight unique concept designs were identified from these methods. These were then evaluated using Pugh Criteria — a ranking system of the relative merits of each concept based on design requirements identified. Four of the eight concepts were chosen to be prototyped: a 3-D printed seat, a swing based template, an acrylic-based support and an adaptable wheelchair. The prototypes were made in collaboration with the UCD School of Engineering and tested using paediatric phantoms in UCD Radiography department. The final prototypes will be trialled in Crumlin Children's Hospital with a view to future use and development.

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◆ *Thrombotic Microangiopathy and the Kidney*

Ⓟ AM Dorman

Beaumont/RCSI, Dublin, Ireland

Thrombotic microangiopathy (TMA) is a pathology that results in thrombosis of capillaries and arterioles due to endothelial injury. It is usually characterized by an atypical haemolytic syndrome (aHUS) or thrombotic thrombocytopaenic purpura (TTP). TMA is considered to be caused by infections, drugs, autoimmunity, tumours, pregnancy, transplants and inherited abnormalities involving the alternate complement pathway. This presentation describes the pathology of TMA. It includes a retrospective 15 year study (1999 - 2013) of all renal biopsies reported by one pathologist. All renal biopsy request forms and reports, where cases included light (LM), fluorescence(FM) and electron microscopy(EM), were reviewed. Cases without all 3 modalitles (LM, FM, and EM) were excluded. 6639 biopsies were reported in the study period (328 in 1999 to 629 IN 2013). 2105 were transplant biopsies. 284 biopsies were insufficient (LM, FM and EM all not possible. This resulted in 4250 native renal biopsies as the study group. Following review of the reports 641 cases were reported as TMA. 66 were associated with thin membrane nephropathy, 41 with minimal change disease and 24 with plasma cell dyscrasia/ B cell malignancy. This resulted in 510 cases with TMA as the only pathology reported which represents 12% of all adequate native medical renal biopsies. Clinical indications included proteinuria in 73%, nephrotic syndrome in 15%, increased creatinine in 43%, increased blood pressure in 55% and haematuria in 43% of the cases. Acute renal failure was described in 10% and HUS in just 1% of the cases. Pathological changes were predominently arteriolar sclerosis and glomerular double contours on LM with chronic subendothelial injury on EM. The conclusions of this presentation are 1. TMA is overwhelmingly a chronic lesion as seen in renal biopsy pathology. 2. It is a very common pattern of injury. 3. It is not usually associated with clinical HUS or TTP features at presentation.

◆ Next Generation Sequencing: What Will it Mean for the Pathologist?

Ⓟ CL Corless

Oregon Health & Science University, Portland, Oregon, USA

The era of targeted cancer therapeutics has brought forth new challenges for molecular diagnostic laboratories. The list of genes, and indeed specific mutations, that predict drug responses keeps growing, and with it grows the demand for molecular sub-classification of tumors. In colorectal carcinoma, for example, recent studies support expanding testing beyond KRAS to include NRAS and BRAF in predicting resistance to EGFR-targeted therapies. Similarly, in non-small cell lung carcinoma standard screening for EGFR mutations and ALK gene fusions may be insufficient when actionable alterations involving ROS1, RET, HER2, MET, BRAF and other genes are being targeted (successfully) in ongoing clinical trials. Fortunately, the introduction of next-generation sequencing (NGS) into the clinical laboratory is meeting the demand. Due to its quantitative output, NGS not only provides precise mutant allele ratios, but it can also be used to detect gene gains and losses. Furthermore, when applied to RNA, NGS supports the detection of gene fusions and serves in assessing gene expression levels. While NGS is a powerful tool for molecularly characterizing solid tumors, the quality of the results in large part rests on the selection of appropriate input material; therefore, review by a pathologist prior to testing remains a cornerstone to success. Other growing uses of NGS include monitoring minimal residual disease in the setting of hematologic malignancies, and in the detection of targetable mutations in cell-free DNA within the plasma.

◆ Next Generation Sequencing in Muscle Disease – A Tale of Two Cities

Ⓟ AR Foley

National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, USA

The advent of next generation sequencing has ushered in an era of tremendous potential for identifying the molecular causation of simple and complex disorders, both rare and common. The successes of next generation sequencing reflect the combined interpretive skills of geneticists, bioinformaticians and clinicians working in close collaboration.
In the realm of muscle disease, we have witnessed both the strengths and the weaknesses of next generation sequencing technologies. The use of exome sequencing in patients with rare muscle disease who have been carefully phenotyped has proven to be a successful strategy for identifying causative variants in new genes as well as in known genes. In fact, exome sequencing has significantly expanded both the clinical and the histological phenotypic spectra of muscle conditions associated with causative variants in known genes. In the absence of careful phenotyping or large genetic reference data sets for identifying variants of interest, causative variants may be missed, however. The use of RNA sequencing- using RNA extracted from muscle biopsy specimens- has proven to be a powerful tool for finding causative variants affecting gene splicing or expression, which may be missed with next generation sequencing. Muscle pathology plays an essential role in complementing next generation sequencing. The deep phenotyping of patients with muscle disease relies heavily on muscle histological and immunohistochemical findings in combination with muscle imaging, clinical history and neuromuscular examination findings. Examples of how next generation sequencing coupled with careful clinical and histological phenotyping has uncovered causative variants in new genes as well as in known genes will be discussed in this talk.

◆ Companion Diagnostics: The Evolving Role of Diagnostic Pathology

Ⓟ MTP Padilla

Ventana Medical Systems, Inc./Roche Tissue Diagnostics Companion Diagnostics, Tucson, USA

Medicine, diagnostic pathology, technology, and diagnostic tests are evolving at an extremely rapid pace. Drug developers and diagnostic developers each face unique challenges. Companion diagnostic development is a key component of pharma drug development strategy. We will discuss the importance of companion diagnostics in the success of personalized medicine, the FDA position on companion diagnostics, and the role, advantages and disadvantages of tissue based companion diagnostics. Other technologies, such as Next Gen Sequencing, will increasingly be utilized in a complementary fashion along with traditional slide based immunohistochemical and in situ hybridization. The scope and limitations of available technologies will be reviewed. Diagnostic technologies of all types will complement each other to provide the most accurate diagnostic information for clinicians and patients.

◆ Companion Diagnostics for Haematological Malignancies

Ⓟ RJ Flavin

St. James's Hospital, Dublin, Ireland

Following the 2008 WHO classification of haematological malignancies there has been a greater emphasis on the integration of molecular information with clinical and morphological data not just for diagnostic purposes but also to help convey both prognostic and therapeutic information. This talk will concentrate on routine testing in the work-up of common haematological malignancies focusing specifically on clonality and translocation analysis in lymphoproliferations and mutational testing in BCR-ABL negative myeloproliferative neoplasms. Using case studies to illustrate common indications for testing this talk will also highlight some of the practical points and pitfalls in the interpretation of these tests.

◆ The Neuropathology Post Mortem for Trainees

Ⓟ M Farrell

Beaumont Hospital, Dublin, Ireland

"Should I keep the brain?" is one of the most frequent questions addressed to neuropathologists by surgical pathology colleagues. Fears relating to inappropriate organ retention coupled with decreasing availability of expert neuropathology opinion and the widely held belief that advances in neuroimaging have replaced the brain autopsy, have all contributed to a decline in the post mortem study of human brain tissue. Leaving aside the critical relevance of neuropathology to forensic medicine, the vital role played by careful examination of the post mortem brain extends far beyond pathology and has contributed greatly to science and medicine. In general, prolonged retention of entire brains may be avoided. In hospital practice it is uncommon for a patient to die without brain imaging. Access to pre-mortem brain imaging will guide the surgical pathologist in careful and appropriate sampling of calvarial, dural, meningeal, vascular and parenchymal central nervous system components. Spinal cord examination requires prior experience in spinal cord removal but most post mortem technologists are expert in cord extraction. Sampling and appropriate processing of nerve and muscle requires prior experience or neuropathology advice. High quality photography obtained at all phases of post mortem brain examination including the coronally sectioned individual cerebral hemispheres, with retention of blocks from each of the brain lobes together with cerebellum, brain stem, vessels and dura — meninges will ensure that in the event of a neuropathology opinion benign required — that opinion will not be compromised. Specific issues which will be addressed will include the death of patients with epilepsy, dementia, stroke and undiagnosed neurological disease. The key learning objective will be to ensure that pathology trainees approach post mortem examination of the nervous system with interest and excitement.

◆ Approach to the Neuropathology of Brain Tumours

Ⓟ KM Kurian

Institute of Clinical Neurosciences, Bristol, UK

Dr Kathreena Kurian is a Consultant Neuropathologist and Head of the Brain Tumour Research Group, Institute of Clinical Neurosciences, Bristol. She will present an Approach to the Neuropathological Diagnosis of Brain Tumours and update on what's new in brain tumour research. Kathreena has published widely on Brain Tumour Research and has authored the textbook An Atlas of Gross Neuropathology Kurian KM, Moss T, Camelo-Piragua A; published by Cambridge University Press. She sits on the NCRI Brain Tumour Clinical Subgroup and Novel Agents and Translational Research Subgroup, and is Associate Editor for Frontiers in Oncology and is on the Editorial Board for the Journal of Pathology. She recently launched a National Campaign with the charity Brainstrust to raise awareness among Brain Tumour Patients of the need for consent to use tissue for research .

◆ A National Framework for Quality Assurance in Cellular Pathology – The Irish Approach

Ⓟ N Swan[1]; J O'Keane[2]; K Sheahan[1]; J McCarthy[3]; A Treacy[4]; S Phelan[5]

[1]St. Vincent's University Hospital, Dublin, Ireland; [2]Mater Misericordiae Hospital, Dublin, Ireland; [3]Cork University Hospital, Cork, Ireland; [4]Mater Private Hospital, Dublin, Ireland; [5]Galway University Hospital, Galway, Ireland

Following several high profile misdiagnoses in Ireland a national quality assurance (QA) programme for cellular pathology in 2009 was initiated with a vision of establishing a patient-centred pathologist-led framework that would enhance the quality of patient care with timely, accurate and complete pathological diagnoses and reporting. National QA guidelines were developed based on 17 key quality activities generating a total of 51 Key Quality Indicators (KQI). Examples of the quality activities include turnaround time, monitoring of amended reports, frozen section correlation and various elements of peer review. All 25 cellular pathology departments in the public state-funded hospitals participate in the programme in addition to 7 laboratories within privately-run hospitals. Each laboratory enters codes on individual cases designed to capture the relevant KQI and the anonymised encrypted QA data is then electronically extracted from the laboratory information system to a national database. This national central database is managed by a novel information technology system, the National Quality Assurance Intelligence System (NQAIS)-Histopathology, that was designed to process and display the QA data so that each individual laboratory can analyse their own data and also compare their performance to the national average for each KQI. Since 2013 complete national data has been inputted into the NQAIS system and in 2014 initial QA targets were agreed for turnaround time, frozen section correlation and rate of intra-departmental consultation (IDC). In 2015 additional targets for autopsy ICD, frozen section deferral rate and turnaround time have been added. To our knowledge this programme has enabled Ireland to be the first country to publically report national metrics on the quality of their pathology services.

◆ The Politics of EQA: The NHS England QA Review and its Consequences

Ⓟ DE Hughes

Royal Hallamshire Hospital, Sheffield, UK

In 2013, a review of External Quality Assessment processes was carried out on behalf of NHS England. The main recommendations of this review related to the strengthening of governance of EQA, both nationally and within pathology provider organisations. Recommendations specifically affecting Cellular Pathology were: (i) Professional bodies, led by RCPath, should develop methodologies for assessing the performance of individuals in EQA schemes. (ii) All pathologists reporting pathology results and providing clinical advice should be participate in EQA schemes relevant to their practice, should achieve levels of performance determined by the professional bodies and this performance should be noted at annual appraisal. (iii) Where a need to improve performance is identified, additional remedial training should be carried out, or practice in the area of concern should be stopped until appropriate retraining has been undertaken and revalidation achieved. This process should supported and resourced by the employing organisation, as should EQA scheme participation. (iv) Interpretative EQA schemes are designed to assess and improve individual performance, and attempts at collusion are considered matters of professional probity. The professional response to these recommendations is expected to be led by the RCPath under the guidance of a newly-established national oversight group working on behalf of NHS England and should be more clear at the time of the Pathsoc conference. A key part of this response will be to separately consider the implications of this review for technical schemes, affecting laboratories, and interpretative schemes, affecting individual practitioners.

◆ The Role of Quality Assurance in the Molecular Laboratory

Ⓟ EA Sheppard

Roche Tissue Diagnostics, Tucson, Arizona, USA

Tumour samples to guide treatment decisions have become of increasing significance. Most importantly the results of companion diagnostic testing directly influences the management of individual patients as more drugs are approved for treatment of specific molecular distinct subgroups. Reporting suboptimal quality test results may be harmful to the patient and cause the mismanagement of a prescribed companion drug. The consequences of unsatisfactory performance and measures for improvement are the responsibility of the laboratory. Presently, there are number of EQA schemes for molecular testing available in Europe however, their results clearly indicate the need for EQA since 10%—15% of laboratories do not carry out according to the standard set by the EQA provider or utilize standardized procedures. Continual improvement programs, internal quality control and validation program assist laboratories however; by using standardized quality practices such as ISO 15189 and the use of external quality assurance schemes can provide essential feedback to the laboratory to assure accurate molecular testing results.

◆ How to Run a Histopathology EQA in the Digital Age

Ⓟ NJ Mayer[1];Ⓟ JD Oxley[2]

[1]Cork University Hospital, Cork, Ireland; [2]Southmead Hospital, Bristol, UK

Interpretative EQA schemes in Histopathology were first introduced in the UK in the mid-1980s, well before the advent of the Internet and high resolution digital images. In this lecture we will outline key developments, as EQA schemes have evolved into the digital era, with particular emphasis on the National Urological EQA scheme, which we have run since 2007. We will outline the main practical issues involved in running an EQA scheme and share our personal experience of the development and introduction of the web-based EQAlite software, which is being utilised by increasing numbers of schemes. We will address the pros and cons of traditional glass slide-based circulations versus virtual circulations using scanned digital images and show how the digital archive generated from old EQA circulations has become a valuable educational and teaching resource. We will also briefly explore, from an Organiser's perspective, the major issues facing EQA schemes in the future, as EQA performance becomes more embedded into revalidation and fitness to practice.

◆ Molecular Pathology: The Future?

Ⓟ CS Herrington

University of Edinburgh, Edinburgh, UK

Molecular pathology is already central to stratified medicine. And the ability of pathologists to understand disease phenotype is essential for interpretation of the current explosion in '-omics' data. Moreover, the future of stratified medicine will require integration of information from different sources, in the context of disease phenotype, to inform patient management: pathologists are ideally placed to lead this integration. This applies not only to data derived from *ex vivo* cells and tissues but also to molecular imaging data, which require accurate correlation with cell and tissue phenotype for accurate interpretation. Molecular pathology is key to the future of pathology; and this future extends beyond the traditional light microscope

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

SOCS2 as a Marker Related to Low Grade and Tumour Morphology in Breast Cancer

Ⓟ M Craze[1]; C Joseph[1]; R Russel[2]; OM Rueda[2]; E Provenzano[3]; C Caldas[4]; IO Ellis[1]; A Mukherjee[1]

[1]University of Nottingham, Nottingham, UK; [2]CRUK Cambridge Research Institute, Cambridge, UK; [3]Addenbrooke's Hospital, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK; [4]CRUK Cambridge Research Institute, Addenbrooke's Hospital, Cambridge, UK

Hypothesis: Suppressor of cytokine signalling (SOCS) family members play a vital role in the activation of the JAK/STAT signalling pathway via a negative feedback loop and have been implicated in the development of cancers. In breast cancer (BC), SOCS2 mRNA has been correlated with oestrogen receptor (ER) positive tumours favouring a good prognosis (BMC Cancer 2007, 7:136). This study aimed to determine whether SOCS2 at the protein level correlates with tumour morphology and low grade in BC.
Methods: Differential expression analysis between tubular and grade matched NSTs were undertaken in the METABRIC cohort. Primary breast cancer tissue microarrays (n=1041) were immuno-stained for SOCS2 and expression patterns correlated with clinico-pathological and molecular variables including outcome.
Results: Differential gene expression analysis on the METABRIC data identified SOCS2 as the top gene with a significant overexpression in the tubular type as compared to low grade NSTs (adjusted p value=0.004). Immunohistochemistry on the Tenovus series showed positive nuclear SOCS2 expression to correlate with tumours of low grade ($p<0.0001$), low proliferation (Ki67 $p<0.0001$), ER/PR positive ($p<0.0001$) phenotype and tubular morphology ($p<0.0001$); as well as negative HER2 status ($p=0.005$) and non-triple negative status ($p<0.0001$). Survival analysis revealed significant associations with long term breast cancer specific survival ($p=0.019$). Positive SOCS2 correlations were also observed with the expression of androgen receptor (AR) ($p<0.0001$) and STAT3 ($p=0.001$), further indicating its role in these two signalling pathways.
Conclusions: Results from this study suggest SOCS2 to be a marker of favourable prognosis: identifying low grade, ER positive breast tumours with particular correlations to the tubular histological tumour type.
Project supported by Career Development Fellowship from PathSoc and NIHR

Novel Hypoxia-Associated Markers of Chemoresistance in High Grade Serous Ovarian Cancer

L McEvoy[1];Ⓟ SA O'Toole[1]; CD Spillane[1]; B Stordal[1]; M Gallagher[1]; CM Martin[1]; L Norris[1]; N Gleeson[1]; A McGoldrick[2]; F Furlong[3]; A McCann[2]; O Sheils[1]; JJ O'Leary[1]

[1]Trinity College Dublin, Dublin, Ireland; [2]University College Dublin, Dublin, Ireland; [3]Queens University Belfast, Belfast, UK

Background: Ovarian cancer is the fifth leading cause of cancer in women and has poor long-term survival, in part, due to chemoresistance. Tumour hypoxia is associated with chemoresistance in ovarian cancer. However, relatively little is known about the genes activated in ovarian cancer which cause chemoresistance due to hypoxia. This study aimed to firstly identify genes whose expression is associated with hypoxia-induced chemoresistance, and secondly select hypoxia-associated biomarkers and evaluate their expression in ovarian tumours.
Methods: Cisplatin-sensitive (A2780) and cisplatin-resistant (A2780cis) ovarian cancer cell lines were exposed to combinations of hypoxia and/or cisplatin as part of a matrix designed to reflect clinically relevant scenarios. RNA was extracted and interrogated on Affymetrix Human Gene arrays. Differential gene expression was analysed for cells exposed to hypoxia and/or treated with cisplatin. Potential markers of chemoresistance were selected for evaluation in a cohort of ovarian tumour samples by RT-PCR.
Results: A wide range of genes associated with chemoresistance were differentially expressed in cells exposed to hypoxia and/or cisplatin. Selected genes [ANGPTL4, HER3 and HIF-1α] were chosen for further validation in a cohort of ovarian tumour samples, n=35. High expression of ANGPTL4 trended towards reduced progression-free and overall survival. High expression of HER3 trended to increased progression-free but reduced overall survival, while high expression of HIF-1α trended towards reduced progression-free and increased overall survival.
Conclusion: This study has further characterized the relationship between hypoxia and chemoresistance in an ovarian cancer model. We have also identified many potential biomarkers of hypoxia and platinum resistance and provide initial validation of a subset of these markers in ovarian cancer tissues.

Gene Network Analysis Reveals a Novel Pathological Cell Type in Paediatric Focal Cortical Dysplasia

Ⓟ F Scerif[1]; SR Picker[1]; SA Yasin[1]; A Alahdal[2]; A Virasami[2]; W Harkness[3]; M Tisdall[3]; F Guillemot[4]; SML Paine[1]; JH Cross[1]; TS Jacques[1]

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Introduction: Focal cortical dysplasia (FCD) is a malformation of cortical development that is a frequent cause of multidrug resistant paediatric epilepsy. FCD type IIb is characterised by a population of unique abnormal cells known as balloon cells (BCs). The pathogenesis of FCDIIb is poorly understood and it is unclear if BCs are the key pathological cell or if there are other types of cells that are important in the pathogenesis of the disease.
Methods: Analysis of Affymetrix™ Human Exon 1.0ST microarray data revealed differentially expressed genes (DEGs) between a BC group and a control non-BC group. Ingenuity Pathway Analysis (IPA; bioinformatics software) was used to identify networks of the DEGs. The expression of a micro-network was validated using immunohistochemistry. Double immunofluorescence was undertaken to identify the lineage of cells expressing components of the network.
Results: We identified a network of interacting genes that were upregulated in FCDIIb compared to normally formed cortex or FCD without balloon cells (FCDIIa). Some components of this network were expressed in BCs but others were expressed in novel cell populations. Double immunofluorescence identified a cell with the phenotype of a glial progenitor that was only present in FCDIIb but not in normally formed cortex.
Conclusions: We have identified a novel population of glial progenitors found frequently adjacent to BCs in FCDIIb. Paracrine signaling between BCs and the novel CHI3L1 positive cells is likely to be involved in the pathogenesis in FCDIIb. Further investigations into the role of these cells would give us a better understanding of the molecular abnormalities underlying FCD and possibly provide novel therapeutic targets.

- ***Scerif F, Picker SR, Yasin SA, Virasami A, Alahdal A, Harkness W, Tisdall M, Guillemot F, Paine SML, Cross JH, Jacques TS. Identifying Novel Cell Types in Focal Cortical Dysplasia by Gene Network Analysis. Neuropathology and Applied Neurobiology ∏ 2015 British Neuropathological Society, 41 (Suppl. 1), 30-58.***

Post Mortem Microarray and Methylation Studies in Stillbirths with Unexplained IUGR

Ⓟ IU Nicklaus-Wollenteit[1]; L Cooper-Charles[2]; D McMullan[2]; T Marton[2]; D Lim[2]; L Brueton[2]; P Cox[2]

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Introduction: Intrauterine death (IUD) at ≥24 weeks gestation affects ~1 in 200 pregnancies, with intrauterine growth restriction (IUGR) present in approximately 50%. Although frequently due to placental pathology, genetic abnormalities may also underlie a significant proportion and Silver-Russell syndrome (SRS) may be implicated in some.
Objective: This first comprehensive pathological and genetic study to investigate non-placental IUGR, hypothesises recurring abnormalities common to this group. There is limited experience of these techniques in IUD's. The results of this study will advance the understanding of the genetic influence in the pathogenesis of IUGR and IUD and will inform future routine clinical diagnostic practice to ensure that valuable resources are used in a cost effective manner and may be applicable to prenatal diagnosis in cases of detected IUGR in utero.
Methods: 31 IUD's ≥24 weeks gestation with non-placental IUGR (<3rd centile), with/ without congenital anomalies were selected. Standard microarray and MLPA testing for SRS was carried out. Where normal, a higher resolution microarray containing SNP probes was used to test for smaller imbalances, uniparental disomy 7 (UPD7) or loss of heterozygosity (LOH).
Results: One case showed a homozygous deletion of part of the FANCA gene, consistent with Fanconi anaemia and 3 cases remain as uncertain findings. No cases of SRS were identified. The higher resolution microarray did not identify any smaller imbalances and no case of UPD7. 3 cases showing LOH over a gene associated with IUGR are undergoing mutation screening follow-up. **Conclusion:** The comprehensive genetic study of a representative cohort has not shown a frequently recurring underlying genetic abnormality and suggests that causes of IUGR are complex. Further investigations of the cases with findings of uncertain significance will determine whether these are pathogenic.
Acknowledgement: This project is grant funded by Path Soc.

Advanced Neoplasia Detection in Colorectal Cancer Screening Using Multiple Stool DNA Markers and Haemoglobin

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Purpose of the study: Molecular tests have the potential to improve current non-invasive faecal immunochemical test (FIT) screening for colorectal cancer (CRC) and advanced precancerous lesions. We examined the performance of a panel of faecal DNA (sDNA) markers and FIT in archival samples from an invitational CRC screening population.
Methods: Whole stool samples were prospectively collected from individuals participating an invitational primary colonoscopy-screening program (COCOS trial). Only participants that provided stool, performed FIT (OC-Sensor) and underwent colonoscopy were selected. The sDNA panel included quantitative molecular assays for KRAS mutations and for aberrant NDRG4 and BMP3 methylation. The performance of the sDNA plus FIT panel was compared to the FIT results alone, by Receiver Operator Characteristic (ROC) analyses.
Results: 1047 individuals (51% male) with a median age of 60 years (range 50-75) were included, of which 7 (0.7%) had colorectal cancer and 104 (9.9%) had advanced precancerous lesions (advanced adenomas or sessile serrated polyps ≥ 1 cm). The combination of sDNA and FIT was more sensitive than FIT alone for detecting advanced precancerous lesions (49% (50/102) and 25% (26/102), respectively). Specificities among individuals with non-advanced or negative findings (controls) were 89% and 96% for sDNA and FIT testing, respectively. ROC analysis of CRC and advanced precancerous lesions compared to controls revealed an Area Under the Curve (AUC) of 0.75 for the sDNA plus FIT test, compared to 0.68 for FIT alone. At an equal specificity of 95%, advanced precancerous lesions were detected with higher sensitivity by the sDNA plus FIT test compared to FIT alone (36% vs 28%, p=0.08).
Conclusions: In an invitational colorectal cancer screening cohort, combining stool DNA markers with FIT detected more advanced neoplasia than FIT alone, primarily due to detecting more advanced adenomas.

The Three-Dimensional Anatomy of the Anal Sphincter Complex and its Relevance to Low Rectal and Anal Pathology

AC Kraima[1]; Ⓟ NP West[2]; D Treanor[2]; N Roberts[2]; D Magee[3]; NN Smit[1]; CJH Van de Velde[1]; MC DeRuiter[1]; HJ Rutten[4]; P Quirke[2]

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Excellent anatomical knowledge of the anal sphincter complex (ASC) is essential for the treatment and understanding of low rectal and anal pathology. Some of the current descriptions of the ASC are contradictory. In this study, the three-dimensional (3D) anatomy of the ASC is described with relevance to low rectal and anal surgical pathology.
Six human adult cadaveric specimens (three males, three females) were obtained from the Leeds GIFT Research Tissue Programme. Paraffin embedded mega-blocks containing the ASC were serially sectioned at 250 µm intervals. Sections were stained with haematoxylin & eosin, Masson's trichrome and Millers' elastin, from which 3D reconstructions were developed. The ASC is a complex structure, varying between individuals in the size and distribution of its layers with intermingling of fibres and inconsistency of the longitudinal smooth muscle affecting the creation of the surgical intersphincteric plane. Longitudinal fibres penetrate the internal and external anal sphincter to anchor in the submucosa and ischiorectal fossa. Striated muscle fibres from the external sphincter were identified in the submucosa in four of six specimens. The ASC is highly complex due to the degree of variation in its structure and intermingling of smooth and striated muscle fibres and their penetration of major structures. This creates potential tissue planes for the spread of infection, fistula extension and tumour spread. The complex anatomy of the ASC also impacts on the staging of low rectal cancers in this region, which requires further investigation.

cTEN Regulates Cell Motility through Snail in Colorectal Cancer

Ⓟ H Thorpe; A Asiri; M Akhlaq; D Jackson; M Ilyas

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Cten is upregulated in a number of tumour types and in colorectal cancer expression is associated with advanced Dukes stage, poor prognosis and distant metastasis. Cten is localised at focal adhesions and regulates cell motility but knowledge of underlying signalling mechanisms is sparse. Epithelial to mesenchymal transition (EMT) is a process whereby cells acquire an invasive phenotype to aid cell migration and is found to occur in a number of biological processes including cancer metastasis. We investigated whether Cten increases cell migration through EMT pathways in colorectal cancer. Cten was forcibly expressed in colorectal cell lines and Snail expression determined by qPCR and western blot. The cycloheximide pulse chase assay was used to assess any changes in Snail protein stability. Further to this, the Transwell migration assay was performed to investigate changes in cell motility. Forced expression of Cten was shown to increase Snail protein expression in HCT116 and Caco2 cell lines. There was no change in the level of Snail mRNA suggesting that Cten regulates Snail at a post transcriptional level. Inhibition of protein synthesis confirmed this and showed that Cten regulates the stability of Snail protein. Simultaneous forced expression of Cten and knockdown of Snail demonstrated that this relationship was functionally active. Forced expression of Cten increased cell migration ($p<0.05$) which was subsequently lost when Snail was knocked down ($p<0.001$). We are the first to identify Snail as a downstream target of Cten signalling. This finding advances the understanding of cancer cell motility regulatory networks and further highlights Cten as a potential therapeutic target in colorectal cancer.
Work supported by a Pathological Society grant.

Loss of pTEN Expression is Strongly Associated with the Presence of the BRAF V600E Mutation, and Further Complicates Combination Treatment Strategies for Patients with Advanced Colorectal Cancer

Ⓟ SD Richman[1]; GJ Hemmings[1]; P Chambers[1]; M Taylor[1]; HM Wood[1]; E Tinkler-Hundal[1]; K Southward[1]; JM Foster[2]; A Ouime[2]; KG Spink[2]; P Quirke[1]

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Treatment for advanced colorectal cancer is moving to combination therapies, targeting multiple signalling pathways. Indeed, MRC FOCUS4 has been designed to assess this.
We determined pTEN protein expression, and assessed this in relation to other biomarkers associated with signalling downstream of the epidermal growth factor receptor. Tissue microarrays were constructed from 2 advanced colorectal cancer (aCRC) clinical trials (FOCUS and PICCOLO) for immunohistochemistry (IHC). Mutation status of KRAS, NRAS, PIK3CA and BRAF was assessed by pyrosequencing. Copy number variation was assessed on Oncoscan® FFPE Assay Kit (Affymetrix Inc.). pTEN protein expression was correlated with mutation status, MMR status, primary tumour location and copy number. pTEN protein expression for 1288 patients showed complete loss of expression in 85/787 (10.8%) - FOCUS and 64/501 (12.8%) - PICCOLO. BRAF mutation status was significantly different between the pTEN negative and pTEN positive populations ($p<0.0001$), with significantly more pTEN negative tumours having the BRAF V600E mutation. Loss of pTEN expression correlated with genomic deletions involving the pTEN gene. 20/30 (66%) of pTEN negative tumours exhibited loss of the pTEN region (10q), half of which were focal deletions. Only 54/202 (26.7%) pTEN positive tumours showed deletions of this region, and none were focal events. There was no significant difference in either primary tumour site or MMR status ($p=0.1765$) between the pTEN negative and pTEN positive populations. Signalling pathways do not stand in isolation; they are interlinked in a complex signalling network. Current treatment interventions must target the correct pathway combinations if patients are to benefit from targeted therapy. Our data suggests a subset of patients may require dual AKT and MEK pathway inhibition, in addition to anti-EGFR monoclonal antibody therapy and inhibition of BRAF.

Zonal Differences in PD1 Expression in Centre of Tumour Versus Periphery in Microsatellite Stable and Unstable Colorectal Cancer

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Colorectal cancers (CRC) that show evidence of microsatellite instability (MSI-H) are marked by a high tumour infiltrating lymphocyte (TiL) population which is thought to be prognostic. Programmed cell death 1(PD-1) is a negative regulator of the immune system and targeting the interaction with its ligand PD-L1 offers a potential therapeutic target. We aimed to characterize CD8 and PD-1 expression in both the tumour centre (cT) and tumour periphery (pT) of microsatellite stable (MSS) and unstable CRC.
Methods: Paraffin-embedded tumour blocks were cut at 5um, prepared and stained using specific antibodies for CD8 and PD-1. The pT was defined as the area within a 400x high power field (HPF) from the outline of the tumor. The cT was defined as the area at least one 400x HPF apart from the tumor outline toward centre of the tumor. Images were taken at 40x, 100x, 200x and 400x. Positive cells were averaged across 3 high power fields and classified as high or low positivity.
Results: Forty-two specimens have been analysed to date including 28 MSI-H and 13 MSS tumours. Sixty-eight percent of MSI-H were stage II and 69% of MSS were stage III. In the MSI-H group, a high CD8 count in the cT and pT correlated with and earlier tumour size and stage. PD-1 positivity was seen in 61% of MSI-H cT compared to 0% positivity in the cT of MSS tumours. The periphery of both MSS and MSI-H specimens showed significant PD-1 expression with 71% and 85% of samples showing positivity respectively. There was no association between high or low densities of staining and stage.
Conclusions: Zonal differences exist in the expression of CD8 and PD-1 in microsatellite stable and unstable tumours. A high proportion of MSI-H tumours show PD-1 activity in the centre of the tumour despite an improved prognosis. Further profiling of other T cell populations may help to further understand this expression which may act as a biomarker or provide a therapeutic target

Association of Genomic Aberrations with Disease Recurrence in Stage II and Stage III Colon Cancers

Ⓟ E van den Broek[1]; O Krijgsman[1]; D Sie[1]; MA van de Wiel[1]; EJT Belt[1]; SH den Uil[1]; H Bril[2]; HBA Stockmann[2]; B Carvalho[1]; B Ylstra[1]; GA Meijer[1]; RJA Fijneman[1]

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Biomarkers that are able to distinguish stage II and III colon cancer patients at high risk of developing disease recurrence, who may benefit from adjuvant chemotherapy, are still lacking. Genome-wide profiling of somatic aberrations, including gene point mutations, DNA copy number aberrations (CNA) and structural variants (SV), is expected to provide better insight into the molecular pathology of tumour progression and clinical outcome. Genome-wide analysis of CNAs was performed using high-resolution comparative genomic hybridization for microsatellite stable (MSS) stage II and III primary colon cancer samples (n=114). In addition, the prevalence of genes suffering from CNA-associated chromosomal breaks, indicative for SVs, was determined. The mutation status of commonly affected *APC, TP53, KRAS, PIK3CA, FBXW7, SMAD4, BRAF* and *NRAS* genes was examined for 60 samples using targeted massive parallel sequencing. Associations of genomic aberrations with disease-free survival (DFS) rates were explored by log-rank tests using 10,000 permutations. Disease recurrence and DFS rates differed significantly for several CNA-regions ($P<0.05$). A total of 267 genes were recurrently affected by CNA-associated chromosomal breaks (FDR<0.1), among which 168 genes (66%) that were also identified in a previously analysed cohort of 352 metastatic colorectal cancers. Gene point mutation frequencies were in concordance with literature. In a univariate analysis, none of the individual mutated genes appeared to be significantly associated with DFS. In summary, several associations are found between highly prevalent genomic CNAs and disease recurrence in this cohort of MSS stage II and III colon cancers. Further in-depth analysis is required to unravel underlying biology that contributes to disease recurrence.

Comparison of Histologically Normal Mucosa and Blood as Controls for Targeted Next Generation Sequencing Analysis in Patients with Colon Cancer in the NCRI FOxTROT Trial

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Accurate and reliable methods for assessing the molecular profile of clinical tumour samples are important for the delivery of personalised medicine. When adopting a targeted amplicon sequencing method in combination with next generation sequencing (NGS), it is ideal to call mutations against a control sample to enable artefacts to be removed from the analysis. Blood is considered the gold standard control but may not always be available. We compared the use of histologically normal mucosa to blood as a control in colon cancer. We examined mutations in 40 colon cancers from the NCRI FOxTROT trial using the Fluidigm Access Array for NGS library preparation. We assessed the use of both blood and normal colonic mucosa as a control for assessing mutations in 11 genes. All samples were tested in duplicate. The work was partly funded by a PathSoc Career Development Fellowship and is presented on behalf of the FOxTROT Collaborative Group. Mutation calls made using normal mucosa as a control compared to blood were in good agreement; a Mathew's Correlation Coefficient above 0.7 was seen for all of the genes where agreement could be assessed. We found that false positive mutations were due to poorer amplification of the normal mucosa samples and false negatives were due to mutation calls in the normal mucosa. Overall we found that when assessing mutations in hotspot oncogenes, testing in duplicate and the use of a normal control tissue is not required to make mutation calls. However, where a normal control is required, normal mucosa from the resection margin is a suitable alternative to blood where it is not available.

Sex Cord Tumours Arising in Ovarian and Extraovarian Adenosarcoma: an Unusual Form of Sarcomatous Overgrowth

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We report a series of four unusual ovarian or extraovarian neoplasms composed of an admixture of adenosarcoma and a predominant component comprising a sex cord tumour. The neoplasms occurred in women aged 50 to 69. Three cases arose within the ovary and one was extraovarian (pelvis and abdomen) in location. In all four cases, there were minor areas with morphological features of adenosarcoma with a phyllodes-like architecture and periglandular increased cellularity with mitotic figures. In two cases, the stromal component was morphologically in keeping with a juvenile granulosa cell tumour. In one case, the stromal component had some features of both adult granulosa cell tumour and Sertoli cell tumour within a fibromatous background. The fourth case morphologically could not be categorised as any of the usual types of ovarian sex cord tumour and was categorised as an unclassifiable sex cord tumour. In all four cases, there was immunohistochemical evidence of sex cord differentiation. In each case, we propose that the sex cord tumour arose from a pre-existing adenosarcoma thus representing an unusual form of sarcomatous overgrowth of sex cord elements which can occur within adenosarcomas. This phenomenon is not well described in the literature.

Utility of Serum HE4 in Diagnosis and Prognosis of Endometrial Cancer

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Background: Human epididymis protein 4 (HE4) is a secreted protein that is overexpressed in some cancers. HE4 is emerging as a useful biomarker in diagnosis and follow-up of endometrial cancers. The aim of this study was to evaluate the potential role of serum HE4 in the diagnosis and management of endometrial cancer.
Methods: Patients undergoing surgery for endometrial disease were recruited into this study and had pre-operative serum samples taken, n=157. Demographic, clinical, radiological and laboratory data were reviewed. HE4 and CA125 serum levels were analysed using the Fujirebio Diagnostic ELISA Kits and results correlated with clinicopathological details. Standard cut-off points of 70 pmol/L for HE4 and 35 U/ml for CA125 were used.
Results: HE4 showed a sensitivity of 64% and specificity of 97.50% for detection of endometrial cancer. CA125 had a very low sensitivity of 14% for endometrial cancer diagnosis. HE4 was elevated in all stages of endometrial cancer and demonstrated the ability to distinguish between benign and malignant groups. HE4 also provided information about myometrial space invasion.
Conclusion: HE4 has a role in endometrial cancer diagnosis and prognosis and has the potential to be used in a screening setting or as a triage marker in the primary care setting. For women diagnosed with endometrial cancer, HE4 has the potential to stratify them into treatment regimens where the most appropriate treatment can be delivered resulting in improved quality of life and outcome for endometrial cancer patients.

Platelets Drive Metastatic Changes in Ovarian Cancer Cells

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Background: Ovarian cancer is the 5th leading cause of cancer related deaths in women. Previously we described a dynamic interaction between ovarian cancer cells and platelets in vitro, involving platelet adhesion, activation and induction of pro-survival and pro-angiogenic signals in the cancer cells. This study looked to further investigate this phenomenon in ovarian cancer cells by assessing the molecular changes it induced.
Methods: Cell lines 59M and SKOV3 were used as in vitro models of metastatic ovarian cancer. Platelet cloaking of cells was quantified by flow cytometry. Cells co-cultured with/without platelets for 24hrs were examined by RT-PCR for EMT related changes and by Affymetrix Gene2.0ST arrays for whole transcriptome changes.
Results: Significantly more platelets adhered to SKOV3 cells than 59M cells. While there were different rates of adhesion, the platelets induced similar changes in EMT related genes in both. There was a significant loss in expression of epithelial genes and an increase in mesenchymal genes, indicating the induction of EMT. Whole transcriptome analysis showed that there were a greater number of gene expression changes occurring in SKOV3 cells compared to 59M cells, correlating with the adhesion data. A 32 gene panel of commonly affected genes in both cell lines was identified, many of which form part of an interlinking pathway that is regulated by TGFβ1 and associated with cell adhesion/ECM remodelling. Though only 32 genes overlapped, the biological processes affected in both cell lines were very similar, with 103 of the 148 processes enriched in the 59M data set also seen in the SKOV3 data set.
Conclusion: This study shows that platelets can enhance the metastatic potential of ovarian cancer cells through the induction of EMT and ECM changes. In addition, it has identified a set of 32 genes that hold potential to be in vivo markers of this interaction.

Platelet Cloaked Tumour Cells Suppress NK Cell Immune Surveillance

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Background: During the metastatic cascade, circulating tumour cells rapidly and efficiently adopt a platelet cloak. Platelet cloaking of tumour cells promotes metastatic disease by promoting cellular proliferation, angiogenesis and EMT while inhibiting autophagy and apoptosis. The aim of this study is to examine whether the platelet cloak contributes to tumour cell evasion of NK cell mediated immune surveillance.
Methods: Freshly isolated PBMCs were harvested from healthy donors and stimulated for 18 hours with IL-2 (500U/mL). PBMCs were co-incubated with ovarian (59M and SKOV3), melanoma (Sk-Mel-28) and CML (K562) cell lines that were either uncloaked, or cloaked with washed platelets from healthy donors. The NK-tumour cell receptor ligand systems, NKG2D-MICA/MICB and CD96/CD226-CD155 were examined using NK cell CD107a expression and interferon-gamma production to quantify NK cell mediated recognition and 'killing' of cancer cells.
Results: We first demonstrated that ovarian and melanoma cancer cell lines when cloaked with washed platelets strongly inhibited NK cell antitumor reactivity. Platelet cloaking induced down-regulation of the stress ligands MICA and MICB on the tumour cell coupled with their release into the microenvironment, a known NK cell immune decoy strategy. In addition, platelets significantly down-regulated both CD96 (NK cell) and CD155 (tumour cell), inhibiting NK cell activity. Both mechanisms occur in tandem to comprehensively incapacitate NK cells and promote tumour immune evasion.
Conclusions: Ovarian and melanoma tumour cells are efficiently cloaked by platelets, which facilitates immune evasion by actively suppressing NK cell cytotoxicity and cytokine production.

Role of IGF-IIR/Man-6-P in Glioblastoma Angiogenesis

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Purpose of the study: Glioblastomas (GBM) are the most common and most aggressive primary malignant brain tumours in adults. One of their histopathological hallmarks is the microvascular proliferation; these tumours are among the most angiogenic of malignancies by displaying the highest degree of microvascular proliferation. IGFIIR/Man-6-P is a receptor that belongs to the insulin-like growth factor (IGF) system. The involvement of IGF-IIR/Man-6-P in the process of angiogenesis has been postulated in rare earlier studies. To our knowledge, the role of IGF-IIR/Man-6-P in the neovascularisation of human GBM has never been studied.
Methods: IGF-IIR/Man-6-P expression was evaluated in the vascular compartment from 322 human GBM and from 10 normal adult brain samples by means of quantitative immunohistochemistry on tissue microarray sections. *In vitro* cell line experiments were carried out in order to characterise the IGFIIR/Man-6-P role in angiogenesis.
Summary of results: IGF-IIR/Man-6-P was strongly expressed in the cytoplasm of endothelial cells in hyperplastic vessels and exhibited a dot-staining pattern. We found a higher expression of IGF-IIR/Man-6-P in GBM vessels compared to normal brain vessels (p=0.05). Furthermore, preliminary *in vitro* experiments suggest a role of IGF-IIR/Man-6-P in tube formation but not in growth of the EA.hy926 endothelial cell line.
Conclusions: This work shows a possible role of IGFIIR/Man-6-P in the process of neovascularisation of GBM angiogenesis. Additional investigations are required to confirm the role of this receptor as a direct actor of angiogenesis in GBM.

A Novel View of the Temporal Arteries: Using 3D Histological Reconstruction to Study Microvessel Anatomy

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Purpose of the study: Vasa vasorum (VV) are microvessels which supply vessels that cannot be nourished by diffusion from their own lumina. VV are believed to be a key element in the pathogenesis of vascular diseases. A number of different imaging methods have been used to study the VV but there is still no definitive consensus on their structure. The aim was to describe the normal microvessel anatomy of temporal arteries.
Methods: Human temporal artery, obtained following routine biopsy with ethical approval and patient consent. Samples were embedded into paraffin blocks and serially sectioned at 5 micron intervals. Alternate sections were stained with H&E and scanned to create virtual slides. The slides were aligned, VV were segmented (annotated) and iso-surfaced to generate 3D reconstructions.
Summary of results: The reconstruction shows the structural arrangement of the VV as a complex plexus. No connection to the vascular lumen was visualised. In this segment a hierarchical branching structure was not observed. VV were almost exclusively restricted to the adventitia of the vessel wall. Mean ± SD area of the VV (n = 5283) is 2287.23µm2 (±4956.03). The mean ± SD number of vessels per slide is 60.76 (±15.37). These metrics are based on one arterial specimen.
Conclusion: This method allows us to study the three-dimensional spatial relationships of microvessels within arterial specimens. Furthermore, metric data generated in the process can support the 3D images to study the microvasculature. This method will be applied to diseased arteries in future to generate novel hypotheses about the inflammatory process.
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Loss of Expression of BAP1 is a Useful Adjunct Which Strongly Supports the Diagnosis of Mesothelioma in Effusion Cytology

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Purpose of the Study: It is controversial whether mesothelioma can be diagnosed with confidence in effusion cytology and therefore an ancillary marker of malignant mesothelial cells would be clinically valuable. BRCA-1 associated protein (BAP1) is a tumour suppressor gene which shows biallelic inactivation in approximately half of all mesotheliomas. BAP1 expression is commonly lost in mesothelioma. We investigated whether loss of BAP1 expression can be used to support a diagnosis of mesothelioma in effusion cytology.
Methods: Immunohistochemistry (IHC) for BAP1 was performed on cell blocks from effusions associated with confirmed mesothelioma cases, effusions containing mesothelial cell atypia, benign effusions, and effusions from patients with lung adenocarcinoma.
Results: IHC for BAP1 was performed on 75 cases of confirmed mesothelioma. 43 (57.3%) showed negative staining in the presence of an internal positive control. In 57 effusions considered to have atypical mesothelial cells in the absence of definitive diagnosis of mesothelioma, 8 cases demonstrated negative staining for BAP1. On follow up, 6 of these patients received a definitive diagnosis of mesothelioma in the subsequent 14 months (2 were lost to follow up immediately). Only 5 of 100 consecutive benign effusions were interpreted as BAP1 negative. 47 patients with confirmed adenocarcinoma demonstrated positive staining for BAP1.
Conclusion: We conclude that loss of BAP1 expression in effusion cytology is quite specific for mesothelioma. Whilst it is not definitive, it can be used to support the diagnosis of mesothelioma in atypical effusions. We caution that interpretation of BAP1 IHC on cell block may be difficult and that convincing positive staining in non-neoplastic cells is required before atypical cells are considered negative. We also note that BAP1 loss is not a sensitive test and cannot be used to exclude mesothelioma.

The South-East of Scotland Experience on the Molecular Detection of EGFR, KRAS and ALK Mutations in Lung Adenocarcinomas

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The approval of novel targeted treatments for EGFR-positive and ALK-positive non-small cell lung cancer (NSCLC) has led to the increased requirement for mutation testing services in South East of Scotland. EGFR mutations are typically found in females, Asians and never smokers whereas KRAS mutations are associated with smoking. ALK rearrangements are commonly found in younger patients and never smokers. This study aimed to determine the prevalence of EGFR, KRAS and ALK mutations in South East of Scotland and to evaluate our experience in testing of ALK with IHC and FISH. Data of all patients tested were collected retrospectively from clinical records. From January 2011 to May 2014, we reported mutation rates of EGFR, KRAS and ALK to be 10.4% (67/643), 35.8% (86/240) and 2.3% (7/304) respectively. In our cohort, an increase in one pack years of smoking resulted in a decrease in the odds ratio of EGFR-positivity (OR 0.94, 95% CI 0.92 - 0.96, p<0.001). KRAS-positivity was associated with a history of smoking, with rates in both former (OR 6.26, 95% CI 2.00-19.56, p=0.002) and current smokers (OR 6.82, 95% CI 2.18-21.35, p=0.001) significantly higher than in non-smokers. The number of smoking pack years had no influence on the rates of KRAS-positivity. ALK-rearrangements were found to be associated with never smokers (p<0.001) and younger patients (≤50 years old) (p<0.001). To date, no false positives were reported for parallel testing of ALK with IHC and FISH. We observed 100% sensitivity (7 IHC+/7 FISH+) and 96.6% specificity (113 IHC-/117 FISH-) when comparing IHC with FISH. In conclusion, the prevalence of EGFR mutation in South East of Scotland has reflected mutation rates reported in West of Scotland. Our findings further support the use of ALK-IHC as a diagnostic screening tool.

HPV and Cell Cycle Protein Expression in Advanced Penile Carcinoma: Results from the TPF Trial

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Purpose of the Study: The molecular mechanisms of metastasis and progression of penile squamous cell carcinoma (PSCC) are unclear. Nobody, to our knowledge, has investigated the expression of cell-cycle proteins in advanced or metastatic PSCC. We aimed to determine the extent of HPV infection in patients with advanced PSCC and its effect on the expression of the key cell-cycle proteins p53, p16INK4A and retinoblastoma (RB).
Methods: Archival paraffin embedded blocks were obtained from 27 primary penile cancers, all patients having developed locally-advanced or metastatic disease. All patients were treated in the Phase II Trial of docetaxel, cisplatin & 5-fluorouracil (TPF) chemotherapy CRUK/09/001 (Nicholson et al. BJC 2013; 109: 2554-9). Samples were analysed immunohistochemically for p16INK4A, p53 and RB protein expression on a tissue microarray. All tumours were HPV typed using PCR.
Summary of Results: HPV DNA was detected in 8/22 (36%) with HPV 16 present in 7/8 (88%). 5 cases were not suitable for analysis. No association was found between HPV and expression of either p16INK4A (p= 0.3426), p53 (p= 0.1365) or RB (p= 1) using Fisher's exact test.
Conclusions: HPV DNA is detected in less than half of progressive PSCC, suggesting either the loss of HPV in advanced disease or that non-HPV related cancers progress more commonly. The lack of correlation between HPV and these cell-cycle proteins suggests that they may undergo somatic mutation that is not driven by HPV, leading to increased growth and invasiveness. Treatment strategies may be hampered by this genetic diversity, which requires further investigation.

Altered Endosome Biogenesis in Prostate Cancer Has Prognostic Potential

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Prostate cancer is the second most common form of cancer in males, and the incidence of this disease is predicted to double globally by 2030. More than 1.1 million new cases of prostate cancer are diagnosed each year and two thirds of these patients are from the Western world. Current diagnostic tests for prostate cancer are limited in both sensitivity and accuracy, and a method for accurate prognosis in these patients is yet to be developed; therefore, there is a need for a sensitive and specific prostate cancer test to implement early and appropriate therapy. The recent discovery of altered endosomal-lysosomal biogenesis in prostate cancer cells has identified a fundamental change in the cell biology of this cancer that holds great promise for the identification of novel biomarkers that can predict disease outcomes. Investigation of the endosome compartment and endosome biogenesis revealed elevated gene and expression of critical machinery components that are required for endosome biogenesis and endocytosis. Here we demonstrate significantly altered expression of endosomal and lysosomal genes in mRNA microarrays of prostate cancer tissue compared to non-malignant tissue, and that specific endosomal and lysosomal genes are predictive of patient outcomes. Two endosomal tri-gene signatures were identified that had a significant capacity to stratify patient outcomes. Changes in the expression of these genes was further ascertained by qPCR in fresh-frozen prostate tissue specimens, which further implicated altered endosome biology during disease progression, with significant changes in expression observed between aggressive prostate cancer and indolent disease or normal prostate tissue. These findings support the initiation of a retrospective trial to determine if these new biomarkers can accurately predict clinical progression in prostate cancer patients.

Molecular Pathways Involved in Lymphovascular Invasion: A Biomarker Driven Approach

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Introduction: Lymphovascular invasion (LVI) is an important step in the metastatic cascade. Identification of a molecular signature for the LVI positive phenotype will help identify relevant drivers and pathways. This study aimed to investigate determinants of LVI from a biomarker database.
Methods: Biomarkers (n >200) from a well annotated series (n=1929) were analysed for correlations with LVI [clinical/IHC (D2-40) supplemented]. Proteins with significant associations with LVI were interrogated for pathway enrichment analysis [corrected for false discovery rate (FDR)], using the STRING 9.1 platform incorporating Gene Ontology (GO), KEGG and NCI.
Results: Biomarker analysis related to both clinical/IHC determined LVI identified 35 positively associated markers, 14 in both clinical and IHC categories (e.g. ADA3, CD8, FOXP3, KPNA2). A further 21 markers were negatively associated, 8 in both categories (e.g. Bcl2, BRCA1, MAGE3 and SOX10). Significant pathways (p<0.001) unifying the positively associated proteins include metabolism, immune responses (T-cell regulation and differentiation), cell activation and transcription [GO]; T-cell receptor signalling pathways and pathways in cancer and haematopoietic cell lineages [KEGG]. For negatively associated proteins, the following were significant: ubiquitination processes, regulation of the mitosis [GO]; p53 pathways [KEGG] and apoptotic and cell cycle pathways [GO & KEGG]. On cross-validating a subset included in the METABRIC cohort, there were overlapping enrichments for immune response regulation (GO) and haematopoietic cell lineages (KEGG).
Conclusions: These preliminary findings are the first to unify biomarkers for LVI pathway analysis in BC, using protein based data. Within the constraints of selection bias, data mining from immunohistochemistry of multiple biomarkers in relation to biological processes hold promise. *AM supported by the NIHR and the Academy of Medical Sciences

Assessment of HER2 Status on Needle Core Biopsy of Breast Cancer: Impact of Histopathological Concordance

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One of the key recommendations introduced in the ASCO/CAP update guideline recommendation on HER2 testing is the novel concept of "histopathological concordance." It is proposed that certain tumour morphological features such as histologic type and grade should trigger repeating a molecular test in cases of "discordance". In this study we have we have reviewed 3104 breast cancer cases consecutively reported in routine practice in Nottingham in the last 4 years. Data on HER2 status was collected and cases with HER2 assessed on resection specimens (RS) were analysed in details.
Results: of all cases, 98 patients (3%) had HER2 status assessed on core biopsy and the corresponding tumour RS. The main reasons for a repeat were tumour multifocality and morphologically different or heterogeneous tumours. A few cases were repeated because of borderline negative FISH results or neoadjuvant therapy. 18 cases were repeated due to insufficient tumour in the core biopsy. In this study the HER2 status of the index tumour was changed in 2 cases and both were in the borderline result category. HER2 testing of different tumour foci of multifocal or morphological heterogeneous tumours was consistent with that of the index tumour assessed on the core biopsy apart from two cases; one positive and one negative. 17 tumours were upgraded from grade 2 on core to grade 3 on excision and HER2 status did not change. No contribution of hormone receptor or tumour type was identified.
Conclusion: There is excellent agreement between HER2 assessed in core biopsy and RS. Histopathological discordance seems to play a minor role which does not justify test repeat in routine practice.

- ***Part of the abstract has been published as part of a letter in the JCO. Rakha EA, Pigera M, Shaaban A, et al: National guidelines and level of evidence: Comments on some of the new recommendations in the American Society of Clinical Oncology/College of American Pathologists human epidermal growth factor receptor 2 guidelines for breast cancer. J Clin Oncol doi: 10.1200/JCO.2014.59.7211***

Molecular Mediators of Mammographic Density

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Purpose of Study: Mammographic density (MD) is a major risk factor for the development of breast cancer though little is known about the biological mechanisms mediating it. Tamoxifen prevents breast cancer in a sub-set of high-risk women in a mechanism that appears to be dependent on reduction of MD. Animal model studies suggest that tamoxifen remodels the mammary stroma to a tumour-inhibitory phenotype. This study aims to analyse the effect of tamoxifen on breast fibroblast function and identify potential pro-tumourigenic pathways contributing to density-associated risk.
Methods: Primary human breast fibroblasts were treated with hydroxytamoxifen (100nm-5μM). Fibroblast function was analysed by measuring: proliferation; expression of stromal proteins fibronectin (FN), LOX and collagen 1; effects on TGF-β signalling via SMAD phosphorylation and upregulation of the myofibroblast marker SMA. Genome wide analysis was performed using RNA-Seq.
Summary of Results: Fibroblasts from 25 patients were treated with tamoxifen. All patients showed reduced proliferation with treatment. In 62% of patients tamoxifen treatment resulted in reduced expression of FN. TGF-β-mediated upregulation of SMA and FN were consistently inhibited by tamoxifen, as was fibroblast contraction of collagen gels. RNA-Seq analysis revealed modulation of a number of metabolic pathways by tamoxifen, including significant upregulation of DHCR7, part of the microsomal antioestrogen binding site (AEBS).
Conclusions: These data indicate that tamoxifen can directly remodel the stromal microenvironment, generating a less 'reactive' stroma. Modulation of AEBS activity has been proposed to be anti-tumourigenic, and also is implicated as a suppressor of Hedgehog signalling. Thus, tamoxifen impacts on multiple pathways to create a tumour inhibitory phenotype.
This work was supported by the Pathsoc Small Grant Scheme.

Mitogen Activated Protein Kinase Signalling Proteins are Associated with Good Prognosis in Breast Cancer and are Mainly Related to Estrogen Receptor

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Purpose of Study: Mitogen Activated Protein Kinases (MAPKs) are three layer signalling transduction molecules that have diverse cellular functions and behaviour in cancer. This study aims to assess the role of a panel of MAPKs biomarkers in breast cancer (BC) and to examine their expression in six BC cell lines.
Methods: Reverse Phase Protein Array (RPPA) was applied to quantify protein expression of MAPKs (15 biomarkers as total and phosphorylated forms) in six BC cell lines with different phenotypes including estrogen receptor (ER)-/+, HER2-/+ and HER2 transfected cells.
Summary of Results: A strong correlations were observed among different proteins involved in MAPKs pathway. MAPKs proteins showed associations with ER status and their differential expression was different between ER-positive and ER-negative cell lines. Importantly, associations between MAPKs proteins and HER2 status (wild and transfected) was mainly seen in the ER negative cell lines.
Conclusions: This study revealed that the high throughput technique of RPPA is useful in testing a panel of biomarkers involved complex biological pathways and networks. MAPKs are mainly related to ER and their association with HER2 was restricted to ER negative status.

Cadherin Switch is More Observed in BRCA1 Mutated than the Basal-Like Breast Cancers

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Purpose of the Study: The Phenotypic features of basal like (BL) breast cancer (BC) resemble those occurring in BRCA1-germline mutation carriers. Several lines of evidence suggesting the overall tendency of basal-like/triple negative BC to spread through vascular rather than lymphatic routes. The latter has recently been attributed to the activation of cadherin switch, an EMT-like phenomenon, in BLBC. This study aims at studying the cadherin switch expression profile TGFB1, a key EMT-trigger, expression in BRCA1 mutated compared to sporadic BC.
Methods: The expression of E-cadherin, N-cadherin and TGFB1 were studied in a subset of germline BRCA1 mutated BC (n= 47) compared to non-selected cohorts of non-lobular sporadic invasive BLBC (n= 422) and non-basal BC (n=1190) using IHC and TMA.
Summary of results: Compared to sporadic BC, BRCA1 mutated cases were of younger age, more grade 3, with more medullary-like tumours, and more LVI positive. E-cad was significantly less expressed in BRCA1 cases than in the sporadic non-basal and in the BLBC. However, N-cad was not significantly expressed in BRCA1, non-basal, and BLBC. TGFB1 was significantly less expressed in sporadic BC, both non-basal BLBC than BRCA1 mutated BC. E-cad/N-cad combinatorial expression phenotypes were significantly different between BRCA1 mutated and non-basal and BLBC. Higher proportions E-cad-/N-cad+ were significantly observed BLBC than non-basal BC. BRCA1 mutated cases displayed the least E-cad+ expression and the highest E-cad-/N-cad+ in the studied series.
Conclusions: Despite the known similarities between BRCA mutated and BLBC, results of this study demonstrate the more occurrence of cadherin switch in BRCA1 mutated breast cancer. E-cad repression appears to contribute more than N-cad gain in BLBC than non-basal BC.

Exploring Molecular Mechanisms Underlying Lymphovascular Invasion in Breast Cancer

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Purpose of the study: Lymphovascular Invasion (LVI) is a crucial step in the metastatic cascade in breast cancer (BC) and is associated with poor prognosis. This study investigated the molecular mechanisms associated with LVI interrogating subsets of the METABRIC series.
Methods: Histological/ immunohistochemistry (D2-40) supplemented LVI were determined in subsets of the METABRIC BC cohort. Cases were stratified into LVI+ and LVI- subgroups. Genes correlating with LVI status were identified in both test (n=179) and validation (n=356) sets from expression profiles using Linear Models for Microarray (LIMMA) data analyses. Biological functions of differentially expressed genes and relevant pathways were explored on multiple platforms.
Summary of results: Initial analysis identified 34362 genes differentially expressed between LVI subgroups. 915 (adjusted p<0.05) overlapping transcripts were identified from test and validation sets, some of which have not been previously linked with LVI. Examples of overlapping genes include APPL1, AQR, CD46, CUL4A, MCFD2, PAPOLA, POT1, RANBP2, SNX4, SUMO1, TLK1, ZNF181/644, SNAP23 etc. Biological function/pathway analysis reveals clusters regulating invasion, transcription, immune-regulation, protein binding and catalytic functions. For example, the proteolysis related SUMO pathway was enriched in both subsets.
Conclusions: Global expression profiling combined with robust histopathological characterisation provides a useful platform to decipher molecular pathways relevant to LVI. Further identification of driver genes associated with LVI is underway combining RNA expression with corresponding copy number alterations, followed by functional analysis.
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Role of Insulin like Growth Factor Binding Proteins and Tamoxifen Resistance in Breast Cancer Epithelial Cells

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The development of tamoxifen resistance (TR) in oestrogen-dependent breast cancer (BC) is a therapeutic challenge. Insulin-like growth factor binding proteins (IGFBPs) may play a role in this process. We have investigated the role of IGFBP proteins in TR BC. IGF axis genes were evaluated in MCF-7 (wt) cells and tamoxifen-resistant (TamR) variants using qRT-PCR and confirmed by ELISA, Western, and Ligand blotting. IGFBP-2 & -5 were knocked down by shRNA transfection, and subsequent sensitivity to 4-hydroxytamoxifen (4-HT) was determined via WST-1. Cell migration was investigated by using the Incucyte system. IGFBP-2 expression was evaluated in 424 BC cases by TMA immunohistochemistry. Five out of 10 genes of the IGF axis (IGF-IR, IGF-2R, IGFBP-2, -4 and -5) had the highest expression levels by both parental wt and TamR cells. IGFBP-5 was down-regulated by ~7-fold while IGFBP-2 was up-regulated by ~2-fold in TamR versus wt cells (mRNA and protein levels). Significantly, a knockdown of IGFBP-2 in TamR cells restored sensitivity to (4-HT), reduced ERα expression to 45 ± 11.9% and enhanced cell migration. Expression of IGFBP-2 was significantly (P< 0.001) associated with survival advantage in TR patients. IGFBP-2 and IGFPB-5 are reciprocally regulated in the acquisition of TR by MCF-7 cells. IGFBP-2 may play a role in the development of TR in vitro and its high levels in clinical samples may predict TR.

Exome Sequencing of Invasive Breast Cancer Specimens Identifies Discordant Mutational Evolutionary Changes in Invasive Primary Tumour and Axillary Nodal Metastases

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Purpose of the Study: Several lines of evidence are currently suggesting that the morphologic heterogeneity of breast cancer is mirrored at the genetic level. Understanding the molecular genetic evolution of BC would contribute further insights into the molecular derangements driving disease progression. Moreover, varied clinical outcome and response to similar therapeutic regimen is attributed, at least in-part to intratumoural heterogeneity. NGS can reliably study the genetic events using miniscule amounts of genomic DNA.
Methods: gDNA was extracted from FFPE tissue sections from a case of invasive duct carcinoma (3 primary tumour samples and 3 samples from positive axillary lymph node metastases). Sample preparation and exome enrichment was performed using Nextera Rapid Capture exome kits (illumina, FC-140-1000). Exome sequencing was performed using illumina MiSeq with 15x depth of coverage (following adapter/barcode trimming). Exploratory analyses and data mining were executed regarding variant (s) concordance/ discordance between primary tumour samples and their respective metastatic variants.
Summary of Results: Initial findings revealed 37 candidate indels common to all three axillary lymph node samples yet absent from the three primary tumour samples. Several genes have been identified as having frameshift mutations caused by indels. Molecular players previously linked to anti-angiogenesis are amongst the genes affected by indel mutations in their coding sequences that may lead to potential abrogation of protein function.
Conclusions: These initial findings provide the framework for detailed molecular analyses for assessing molecular evolutionary events in primary breast cancer and their corresponding metastases.

c-Myc Function is Associated with Specific Molecular Subtypes of Breast Cancer and Confers Resistance to Endocrine Therapy but not Chemotherapy

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C-MYC is amplified in approximately 15% of breast cancers (BC) and is associated with poor outcome. c-Myc protein is multi-faceted and participates in many aspects of cellular function and is linked with therapeutic response in BC. We hypothesised that the functional role of c-Myc differs between molecular subtypes of BC. We therefore investigated the correlation between c-Myc protein expression and other proteins involved in cell cycle control, proliferation, apoptosis and DNA damage together with clinicopathological parameters, outcome and treatments in early invasive primary BC (n=1,106) using immunuohistochemistry. The METABRIC BC cohort (n=1,980) was evaluated for c-Myc mRNA expression. In whole series, there was significant association between c-Myc protein expression with higher tumour grade, lymph node(LN) positivity and medullary-like tumours. C-myc showed differential association with other proteins in the molecular classes. In luminal A tumours, c-Myc was associated with ATM (p=0.005), Cyclin B1 (p=0.002), PIK3CA (p=0.009) and Ki67 (p<0.001). In contrast, in basal-like tumours, c-Myc showed positive associated with Cyclin E (p=0.003) and p16 (p=0.042) expression. c-Myc was an independent predictor of a shorter distant metastases free survival in luminal A LN+ tumours treated with endocrine therapy (ET; p=0.013). c-Myc expression did not predict patient outcome in the other molecular subtypes with respect to adjuvant treatment. High c-Myc mRNA expression was associated with higher grade and basal phenotype (p<0.001). In luminal tumours treated with ET, c-Myc mRNA expression was associated with BC specific survival (p=0.001). c-Myc function is associated with specific molecular subtypes of BC and confers resistance to ET. The diverse mechanisms of c-Myc function, particularly in luminal A BC, warrants further investigation.

Metasin Axillary Predictive Score (MAPS): A Measure of Axillary Nodal Disease Prediction to Provide an Informed Choice for Breast Cancer Patients and Surgeons

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Purpose of study: Intra-operative sentinel lymph node sampling and molecular analysis empowers the surgeon to carry out axillary clearance as a one-step process. We have recently completed the clinical validation of Metasin, an intraoperative molecular assay for sentinel lymph node analysis in breast cancer patients (1836 cases).
Method: The assay uses 2 positive predictive markers and is quantitative, enabling the prediction of tumour volume using 2 markers CK19 and Mammaglobin. In this study group, 439 patients had positive sentinel nodes and 444 cases underwent axillary clearance. Of the axillary clearance cases, 26% contained positive lymph nodes. 84% were sentinel node (SNB) macrometastases, 5% were SNB micrometastases and 11% were SNB negative or contained isolated tumour cells. Informative data was available for sentinel nodes from 125 positive cases.
Results: Using the qPCR values (from Metasin assays using standardised pre-mixes) and clinical axillary clearance data, the cases have been stratified on the basis of the involvement of other axillary nodes. We have shown a three-tiered predictive grouping exists: Group A includes low tumour volume disease with a nodal positivity of 25% within the axilla (n=16): Group B with a 44% positivity of other nodal involvement (n=80) and Group C with positivity of 73% of axillary clearances (n=29).
Conclusion: The clustering of the Metasin data is dependent on the qPCR results and shows that the cases can be sub-grouped to provide a probability basis for prediction of axillary nodal involvement; dependent on the qPCR cut offs. This gives the patient and surgeon a statistical basis for determining the likelihood of other axillary nodal disease.

External Quality Assessment of BRCA1 and BRCA2 Gene Sequencing: Challenges for Quality in a Changing Diagnostic Environment

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Sequencing of the *BRCA1* and *BRCA2* genes has long been used in genetics laboratories to identify cases of familial breast and ovarian cancer. However, the advent of chemotherapy for ovarian cancer based on PARP inhibitors, which requires the presence of a *BRCA1* or *BRCA2* mutation, is turning this specialist test into a commonly-applied companion diagnostic. At the same time, the introduction of new DNA sequencing technologies is posing challenges even for experienced genetics laboratories. EMQN has been providing EQA of *BRCA1* and *BRCA2* gene sequencing world-wide for 15 years. The rate of serious diagnostic errors has varied from year to year, but the mean has hovered stubbornly around 3%. In EQA, just 3 samples per year are sent out, and the quality and experience of participating laboratories varies greatly. We recently carried out a collaborative study to measure the quality of *BRCA* gene sequencing by traditional and new methods in 20 experienced, expert laboratories from 11 countries. Ten DNA samples (8 with pathogenic mutations, 2 with normal DNA sequence) were sent to each laboratory. Ten labs used next-generation sequencing (NGS) alone, 3 used Sanger sequencing alone, and the others used combinations of Sanger sequencing, NGS, MLPA and other technologies. Seventeen (85%) of labs identified all clinically-significant variants on all 10 samples. Four false negative results were reported by 3 labs. Two were due to deficiencies in the bioinformatics pipeline of the NGS process, while 2 were attributed to a sample swap, and incorrect interpretation of a melting profile. No significant trend was identified with respect to the genotyping accuracy of the different methodologies used. The observed error rate of 2% amongst expert laboratories indicates the complex and challenging nature of this kind of testing. Caution will be required when applying these technologies to sub-optimal FFPE samples in Pathology laboratories.

Good or Bad Sequencing Data? Setting a Benchmark for the Quality of Diagnostic NGS in the Laboratory

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Next Generation Sequencing (NGS) is increasingly being introduced into clinical diagnostic laboratories worldwide. The huge amount of data generated by NGS cannot be duplicated by alternative methods for laboratories to internally validate all results, therefore external assessment of data is required. The UK National External Quality Assessment Scheme (UKNEQAS) for Molecular Genetics and the European Molecular Genetics Quality Network (EMQN) have developed a joint EQA scheme for NGS, with the aims to: (a) assess and improve quality; (b) enable laboratories to benchmark their NGS service against others and against best practice; (c) work towards consistency of reporting clinical results generated by NGS; and (d) contribute towards best practice. EMQN and UKNEQAS offer numerous disease-specific, molecular pathology and technical EQA schemes. The objectives for developing NGS EQA were to make it generic (independent of genes, diseases, platforms, and testing context (e.g,. Somatic, germline etc)) and applicable all users. Two pilot EQAs have been run and 157 labs from 32 countries participated. These labs were sent a genomic DNA sample and asked to sequence either their smallest gene panel or largest single gene which the lab tested, submit technical details, and genotypes at known SNPs. The results were compared against a "consensus EQA genome" established by multiple validations of the DNA. 12187 different genes were tested. Most labs are using small panel of 1-10 genes. 60% of all variants were detected by every lab which tested for them. A detailed summary of the key findings will be presented. Both pilots have proved to be challenging to meet our objectives, however the results have enabled clinical diagnostic labs to start to address the quality of their NGS testing.

CTC-5: A Novel Digital Pathology Approach to Circulating Tumour Cell Characterisation

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Tumours invade the vasculature, which transports circulating tumour cells (CTCs) to distant sites enabling growth of secondary tumours. CTCs hold the potential to monitor: therapeutic response, emergent mutations and act as a screening tool for the early detection of cancer. There are numerous methods to isolate CTCs. Once isolated, EpCAM and/or panCK positivity and CD45 negativity are used to verify CTC status. However, due to the metastasis associated process of Epithelial-Mesenchymal Transition, epithelial markers may be ineffective at identifying all CTCs. To overcome such protein marker based limitations, we have developed a novel staining pipeline (CTC-5) that combines histochemical staining (giemsa) with immunofluorescene (DAPI, EpCAM/panCK, HER3 and CD45) staining and whole slide imaging for robust identification, enumeration and characterisation of CTCs from cancer patients. CTCs are isolated from whole blood using ScreenCell Cyto devices. Cyto devices are then slide mounted, giemsa stained and digitised. Giemsa Staining is washed out and slides are immunofluorescently stained for EpCAM/panCK, CD45, HER3 and counter stained with DAPI. Fluorescently stained slides are digitised. Giemsa stained and four colour immunofluorescent digital slides are processed in silico generating a single z-stacked digital slide for pathological assessment. The CTC-5 staining pipeline has been experimentally validated via CTC characterisation of peripheral blood from Lung, Breast and Ovarian cancer patients, with respect to healthy donor and spiked-in controls. The CTC-5 pipeline overcomes recognised weaknesses in CTC characterisation. Histochemical staining is added to the current gold standard of EpCAM/panCK and CD45 staining, while also preserving a fluorescent channel for assessment of biomarker status (e.g. HER3, apoptosis or platelet cloaking). Such advancements enable robust pathological assessment of CTCs in the clinic.

Histogenic Molecular Mapping (HMM) – A Method for Interrogating Biological Pathways in Tissue Sections

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Thorough interrogation of diseased tissue requires the use of multiple biomarkers in order to investigate biological pathways. Unless fluorescent technology is used, multiple sections are required from each tissue block as each section can only be tested for a limited number of markers. Histogenic Molecular Mapping (HMM) is a technique which used digitized images to evaluate multiple biomarkers. Although each section cut from a block is slightly different from the immediately preceding section, the similarity is sufficient to allow non-linear registration of images of successive sections. If the order is known, multiple sections can be mapped onto each other by registering each with the immediately preceding section. This allows several biomarkers to be mapped into a single "composite" section thereby giving a representation of the pathways activated/expressed in the tissue. We used HMM to investigate the mismatch repair pathway in colorectal cancer. Sequential tissue sections were stained for MLH1, PMS2, MSH2 and MSH6 and then scanned. Bespoke computational algorithms were used for image registration and composite images were binned as either "mismatch repair proficient" or "mismatch repair deficient". Validation of each category could be obtained by quantification of pixels in binarized images or pixel distribution using stereology. Our data show that HMM can be used for interrogating biological pathways in tissue sections and, ultimately, automated diagnosis of disease states.

Personalising Treatment in Locally Advanced Rectal Cancer Using Macrophage Subpopulations to Predict the Degree of Response to Radiotherapy

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Whilst pre-operative radiotherapy is the standard of care in locally advanced rectal cancer (LARC), only half of patients respond. Individualised treatment based on a predictive test could avoid unnecessary radiation exposure in poor responders. Macrophages in the tumour microenvironment with tumoricidal M1 and tumour protective M2 phenotypes could be modulating this response. This study investigated the possible predictive value of M1 and M2 subpopulations in identifying the response to short-course radiotherapy (SCRT). Pre-treatment biopsies and post-treatment resection samples were taken from 29 patients with LARC given SCRT. Dual-staining immunohistochemistry was performed with CD68, HLA-DR (M1 marker), and CD163 (M2 marker). Samples were scored for hot-and-random spots by Nuance software (version 3.0.2) and compared with tumour response measured by reduction in tumour-cell density. The work was partly funded by a PathSoc Career Development Fellowship. Samples showing a low score for HLA-DR positive M1 macrophages exhibited a better response to SCRT with a median 80% reduction in tumour cell density (IQR 47 to 85). Those with a high score exhibited a poor response with only a 20% reduction (IQR 0 to 49, p=0·017). No such trends were observed for CD163+ M2 macrophages. The ratio of HLA—DR+ to CD163+ macrophages for biopsy and resection samples was significantly different showing a drop in the HLA-DR positive macrophages in the resection samples (biopsy median 2·53, IQR 1.98 to 3.08; resection median 1·38, IQR 0.96 to 1.80; p=0·024). Assessment of macrophage subpopulations in pre-treatment biopsies appears to predict the degree of response to SCRT in LARC. Further investigation to validate these findings is now required prior to developing a predictive test for use in routine clinical practice. Patients with a poor predicted response could avoid toxic and costly radiotherapy and undergo alternative strategies including chemotherapy.

An Evaluation of Culture Techniques versus 16S Profiling for Investigation of Antibiotic-Mediated Alteration of Microbiota Populations within a Clinically Reflective In Vitro Model of the Human Gut

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Next-generation sequencing technologies (e.g. 16S profiling) are increasingly used to investigate complex bacterial communities. They have advantages over classical methods, as a significant proportion of bacteria are 'non-culturable'. However, they do not distinguish 'viable' and 'non-viable' populations, which may skew results, particularly following antibiotic exposure. Here we report culture and 16S data from a clinically reflective human gut model, describing changes in the gut microbiota following exposure to multiple antibiotics. A triple-stage chemostat model was inoculated with pooled human faeces from healthy volunteers to establish gut microbiota populations. The model was sequentially exposed to clindamycin (33.9 mg/L, QDS, 7days), vancomycin (125mg/L, QDS, 7days) and fidaxomicin (200 mg/L, BD, 7 days). Specific bacterial populations were enumerated daily on selective agars. Periodically, 16S profiling of gut model samples was performed; DNA was extracted on a QIAXtractor, 16S V4 PCR products were sequenced on an Illumina MiSeq, and resulting data were analysed using QIIME. Both culture and 16S profiling demonstrated marked alterations in gut microbiota populations following antibiotic exposure. For many populations, notably bifidobacteria and enterobacteria, changes seen by culture correlated with 16S profiling. However, as culture describes numerical changes in populations, and 16S profiling describes proportional changes, results are not always directly comparable. 16S profiling greatly increased microbiome coverage, particularly for clostridia. Population diversity (number of observed species and Shannon index) decreased with sequential antibiotic exposure. Use of culture and molecular methods in tandem can greatly increase understanding of changes occurring in complex microbial populations.

Spatial Sampling in Barrett's Oesophagus Shows Clonal Evolution of Oesophageal Adenocarcinoma from Metaplastic Non-Goblet Columnar Epithelium

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Barrett's oesophagus is the erosive replacement of the normal squamous oesophageal lining with a glandular epithelium and is the major precursor of oesophageal adenocarcinoma. Barrett's patients are enrolled into active surveillance programmes in order to detect and treat oesophageal cancer at an early stage. Surveillance however is costly and burdening to patients. To improve screening efficacy there is an acute need for accurate biomarkers of cancer progression risk in Barrett's patients. Understanding the pattern and pace of clonal evolution that occurs within the Barrett's segment is a key step towards achieving this goal. Opinion is divided over whether goblet cells (intestinal metaplasia) on oesophageal biopsy are required for a diagnosis of Barrett's oesophagus. This is based on the unproven assumption that goblet cell differentiation marks increased cancer risk in Barrett's oesophagus patients. We have investigated the clonal structure of non-dysplastic and neoplastic Barrett's oesophagus by combining state-of-the-art 3D modeling and genetic lineage tracing. By tracing the clonal origin of an early oesophageal adenocarcinoma through whole-exome sequencing and mitochondrial DNA sequencing, we find that this cancer developed from non-goblet columnar epithelium, whereas the adjacent goblet-bearing mucosa was free of oncogenic mutations. Our results have important implications for the harmonization of the clinical diagnosis of Barrett's oesophagus.

Impact of Neoadjuvant Therapy on Cancer-Associated Fibroblasts in Rectal Cancer

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Purpose of the study: Cancer-associated fibroblasts (CAFs) are increasingly recognised as promoters of tumour progression. It is poorly investigated whether cancer management protocols, such as neoadjuvant radio(chemo)therapy, have an impact on CAFs and, by consequence, on tumour progression. This prompted us to study the impact of neoadjuvant radio(chemo)therapy on the α-SMA/epithelial area ratio in rectal cancer, and the impact of this ratio on recurrence-free survival.
Methods: Immunohistochemistry for the CAF marker α-SMA and the proliferation marker Ki67 was performed on sections from 98 rectal cancers of which 62 had undergone neoadjuvant radio(chemo)therapy.
Summary of results: Computer-assisted quantitative analysis showed that the α-SMA/neoplastic epithelial area ratio was higher after neoadjuvant therapy, and that rectal cancers with high α-SMA/epithelial area ratio had low proliferation rates. Interestingly, the α-SMA/epithelial area ratio was an adverse prognostic factor with regard to recurrence-free survival in univariate analysis. In addition, multivariate analysis showed that an α-SMA/epithelial area ratio above 1 provides an independent prognostic value associated with a poor recurrence-free survival.
Conclusions: These results suggest that neoadjuvant treatment has an impact on CAFs in rectal cancer. The correlation of CAFs with decreased recurrence-free survival and abundant experimental data in the literature suggest that under certain circumstances, not yet very well understood, CAFs may favour tumour progression.

Immunohistochemistry Initiates a Complex Screening Cascade in the Detection of Lynch Syndrome

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Background: Lynch Syndrome (LS) accounts for approximately 2-4% of all colorectal cancers (CRC) and is caused by germline mutations in DNA mismatch repair (MMR) genes. Increasing literature supports routine screening for LS using immunohistochemistry (IHC) to detect loss of MMR protein expression on tumour samples. We reviewed different screening approaches at three National Cancer Centres (NCC) and evaluated the impact on genetic referrals and LS diagnoses.
Methods: CRC databases were analysed from January 2005 - December 2013. NCC1 performs IHC upon physician request; NCC2 implemented reflex IHC (rIHC) in November 2008 and NCC3 has been performing rIHC since 2004. Pathology reports were reviewed and the number of genetic referrals in patients exhibiting MMR-d determined. Patients were also evaluated for BRAF testing and those with positive mutations were not considered eligible for referral to genetics unless otherwise indicated. The number of LS patients detected was calculated as a percentage of the total new patient CRCs.
Results: Over a 9-year period 4,049 new CRC in 3,929 patients were diagnosed across the 3 centres. The implementation of universal screening at NCC3 resulted in a MMR-d detection rate of 11%, an increase of 6% and 8% compared to NCC2 and NCC1 respectively. Referrals to genetic counselling on those patients with MMR-d without BRAF mutations or a known result was low across all centres. The number of LS patients diagnosed did increase from 0.7%(NCC1) and 0.6% (NCC2) to 1.1% at NCC3 however the detection rate remained lower than expected. More than 80% of patients referred, elected to undergo germline testing. Of the LS patients identified 79% had mutations in either MLH1 or MSH2.
Conclusions: The implementation of universal screening using reflex immunohistochemistry detects an appropriate number of MMR-d tumours and increases LS detection rates. However adequate resourcing and clinician awareness are needed to ensure that all patients captured

Residual Tumour Cell Density and the Relationship to Survival Following Pre-Operative Chemoradiation in Locally Advanced Rectal Cancer: Results of the NWCOG RICE Trial

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Pre-operative chemoradiotherapy (CRT) is commonly used to downstage locally advanced rectal cancer (LARC). The degree of response is assessed using a number of subjective tumour regression grading systems. Tumour cell density (TCD) has been developed as an objective linear measure of response and may be more sensitive and reproducible. Patients with MRI-defined LARC received pre-operative CRT using a novel irinotecan-containing regimen, with surgery 9 weeks later. TCD analysis was performed on digitally scanned glass slides. TCD was measured in the pre-treatment biopsy (PTBTCD) and a representative slide from the resection specimen including a 9mm^2 area of greatest TCD (GTCD) and the whole tumour area and/or scar TCD (WTTCD). A systematic sample of 300 random points were inserted into each area using virtual graticule software and manually assessed, TCD was expressed as the percentage of informative points falling on tumour cells. The work is presented on behalf of the NWCOG RICE trial investigators and was part-funded by a PathSoc fellowship. 142 patients commenced CRT and 135 underwent surgery. Median TCD for PTBTCD, GTCD and WTTCD was 38.7%, 7.8% and 1.7% respectively. The number (%) of patients with a TCD of 0% was 0 (0%), 30 (23.6%) and 36 (28.3%) respectively. Distribution of TCD was normal in PTBTCD but highly positively skewed post-resection. Low PTBTCD (split by the median) predicted better 3-year disease free survival (DFS; 76% vs. 60%, p=0.05) although not overall survival (OS; p=0.47). Low WTTCD predicted better DFS and OS (DFS 71% vs. 58%, p=0.05; OS 90% vs. 77%, p=0.02) although no difference was seen for GTCD (p=0.26; p=0.26). Pre-operative CRT markedly reduces TCD in LARC, and provides a continuous measure to compare different regimens. In the pre-treatment biopsy, lower TCD may predict improved DFS. Following resection, TCD across the whole tumour and scar more accurately predicts DFS and OS than using a selected area of greatest TCD.

A Prognostic Classifier for Patients with Colorectal Cancer Liver Metastasis

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Purpose: Clinicopathological scoring systems are currently used to determine prognosis of patients with colorectal cancer liver metastasis (CRCLM). We aimed to establish a prognostic classifier based on biomarkers that reflect tumour biology, to further improve current risk scores.
Methods: Tissue micro-arrays (TMAs) containing formalin-fixed paraffin-embedded tumour specimens of CRCLM and corresponding primary tumours from a multi-institutional cohort of 507 patients who underwent liver resection were immunohistochemically stained for 18 candidate biomarkers. Cross-validated hazard rate ratios (HRRs) for overall survival (OS) and the proportion of HRRs with opposite effect (P(HRR<1) or P(HRR>1)) were calculated. A classifier was constructed by classification and regression tree (CART) analysis and its prognostic value determined by permutation analysis.
Results: Nine of the candidate biomarkers demonstrated putative prognostic value in univariate analysis, and were included in the CART analysis. The resulting classifier was based on AURKA, PTGS2 and MMP9 expression in CRCLM and was associated with OS (HRR 2.79, p<.001), also after multivariate analysis including established clinicopathological prognostic variables (HRR 3.57, p<.001). The prognostic value of the biomarker-based classifier was superior to the prognostic value of the clinicopathological model (p=.001).
Conclusion: A classifier was established for patients with CRCLM with improved prognostic value compared to standard clinicopathological prognostic parameters.

Pathological Response and Specimen Quality Following Long-Course Chemoradiotherapy for Rectal Cancer with a Six vs. Twelve Week Delay: Data From the STARRCAT Randomised Controlled Trial

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Long-course chemoradiotherapy (CRT) is used to down-stage locally-advanced rectal cancer (LARC) prior to resection. An interval period prior to surgery allows for tumour shrinkage to facilitate surgical removal. The optimal time interval remains unclear, with little high-quality evidence to guide clinical decisions about when to operate. This study explores the pathological outcomes from a pilot randomised controlled trial comparing an interval of 6 weeks versus 12 weeks between CRT and surgery. Thirty one patients were recruited from seven UK centres between June 2012 and May 2014. Photographs were taken of the specimens and assessed by a blinded histopathologist for the quality of the mesorectal dissection. Rates of pathological complete response (pCR), down-staging, and circumferential resection margin (CRM) involvement were determined. Response was also assessed using novel tumour cell density (TCD) assessment where the slides from the resected specimen and baseline biopsy were scanned at 400x magnification, the tumour area selected and 285 to 315 data-points analysed by a blinded expert to describe the percentage of different tissue components. The work was partly funded by a PathSoc Career Development Fellowship and is presented on behalf of the STARRCAT Trial Investigators. Twenty three patients underwent surgery (10 from the 6-week arm and 13 from the 12-week arm). The mesorectal fascial plane was intact in 7 specimens from the 6-week arm (70%) and 8 from the 12-week arm (62%). Three patients at 6-weeks and two patients at 12-weeks showed a pCR. Only one patient (from the 12-week arm) had an involved CRM. TCD was 0.3% for the 6-week arm and 4.3% for the 12 week arm (p=0.12). In this small randomised trial, rates of mesorectal quality, CRM status, pCR and TCD were similar following either a 6 or 12 week interval after CRT. Further studies are now needed to clarify whether a longer interval does facilitate on going down-staging.

The Role of Tissue Factor Pathway Inhibitor (TFPI) in Liver Injury

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Introduction: Studies have demonstrated that inhibition of the coagulant cascade is associated with less advanced liver fibrosis and better outcome in acute liver injury. TFPI is a serine protease inhibitor that acts as a homeostatic inhibitor of the coagulation cascade and may be a target to modify outcome in liver disease.
Methods: Transgenic mice carrying a genetic modification that allows cells expressing a-smooth muscle actin (aSMA; e.g. activated hepatic stellate cells) to simultaneously express TFPI were used in models of chronic liver injury (carbon tetrachloride, CCl4) or acute liver injury (paracetamol) and culled at set time points after dosing.
Results: Chronic liver injury: At 24 hours after the last dose of CCl4 the transgenic mice had significantly decreased aSMA expression and tissue inhibitor of metalloproteinase (TIMP) -1 gene expression but no difference in matrix metalloproteinase (MMP) -2 and -9 gene expression compared to wild types. This suggested a microenvironment that would promote fibrosis resolution. However after 24 hours this difference was lost. At all time points there was no significant difference between fibrosis in transgenic and wild type mice as demonstrated by Sirius red staining, hydroxyproline assay and collagen 1a1 gene expression.
Acute liver injury: In paracetamol induced liver injury there was a significant difference in parenchymal necrosis in transgenic mice compared to wild types at 24 and 48 hours after dosing (24 hours: mean necrosis 6% vs. 30% respectively, Mann-Whitney test p=0.008. 48 hours: mean necrosis 2% vs. 20% respectively, Mann-Whitney test p=0.036).
Conclusion: These results suggest that TFPI is an unlikely therapeutic target in chronic liver injury. However in acute paracetamol induced liver injury TFPI appears to rescue the injured liver in a sustained manner from 24 hours after the initial insult and suggests a role for TFPI in managing acute liver injury.
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The Liver Biopsy in Alcoholic Hepatitis: Data from the Steroids or Pentoxifylline in Alcoholic Hepatitis (STOPAH) Clinical Trial

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Introduction: Current guidelines recommend the use of liver biopsy to confirm alcoholic hepatitis (AH) in patients who are clinically classified as severe/high risk. This work sought to validate the Alcoholic Hepatitis Histological Score (AHHS) scoring system and further explore the utility of the liver biopsy in AH.
Methods: Two independent histopathologists, blinded to treatment and outcome, centrally reviewed liver biopsies of patients with clinically high risk AH who had been recruited to the STOPAH trial.
Results: 93/208 (47%) biopsies were both adequate in quality and taken between admission and day 5 of trial treatment. 88% (82/93) had histological features diagnostic of AH. Clinically more severe AH was associated a higher rate of AH diagnosis on biopsy (82% of Glasgow Alcoholic Hepatitis Score [GAHS] ≤ 8 vs. 97% of GAHS >8).
65% (53/82) of biopsy proven cases of AH were classified as severe by AHHS. This group had a significantly higher 28 day mortality rate than those classified as mild/moderate (18% vs. 0%, Fisher's exact p=0.02). AHHS severity positively correlated with baseline Maddrey's Discriminant Function and GAHS (r=0.2, p=0.045 and r=0.3, p=0.01). Clinical markers of severe disease positively correlated with biopsy features of severe disease including serum bilirubin with bilirubinostasis (r=0.5, p=<0.0001) and serum white cell or neutrophil count with lobular inflammation (r=0.4, p=<0.001). However elevation of serum alkaline phosphatase and bilirubin were seen to negatively correlate with ductular change (r=-0.2, p=0.04) and Laennec fibrosis grading (r=-0.3, p=0.01) respectively.
Conclusion: This work goes some way towards validating the AHHS classification. The work also highlights the parallels between clinical and histological parameters and documents negative correlations seen in other liver diseases but not previously noted in AH.

Does EGFR and ALK Mutation Status Correlate With Tumour Morphology in Non-Small Cell Lung Cancer?

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Analysis of adenocarcinoma and non-small cell lung cancer (NOS) for EGFR mutations now forms part of the Royal College of Pathologists' lung cancer dataset. Identification of patients harbouring these mutations facilitates delivery of targeted therapies with superior efficacy. Testing of these tumours for ALK has also been introduced in our centre. We assessed our compliance with the College guidelines in this area for 2013 and 2014. In those tumours positive for EGFR or ALK mutations, we examined the original sections to assess any correlation between mutation status and morphological subtype. 96 of the 116 appropriate cases (83%) diagnosed histologically in 2013 were sent for EGFR mutation analysis, increasing to 183/190 (96%) in 2014. 20 of the cases over this time (7%) were positive for an EGFR mutation. Of these, 12 showed an acinar growth pattern, 3 were solid, 1 lepidic, 1 papillary and 1 micropapillary. It was not possible to characterise the growth pattern in two of the cases analysed as cell blocks. The most common mutation, a missense mutation at codon 858 of exon 21, was most frequently associated with an acinar growth pattern. Of the 2013 cases, 22 (19%) were sent for ALK mutation analysis, compared with 152 (80%) in 2014. Both of the two cases with an ALK translocation (2p23 rearrangement) showed an acinar growth pattern. Our compliance with College guidelines in sending appropriate lung specimens for mutation analysis is improving. The correlations between mutation status and morphological subtype add to, and are in keeping with, the current body of evidence in this area.

Primary Synovial Sarcoma of the Heart – An Interesting Case Report and Review of Literature

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Seventy five percent of primary cardiac tumours are reported to be benign atrial myxomas. The remaining are malignant tumours with most of them being sarcoma, particularly angiosarcoma and malignant fibrous histiocytoma. Synovial sarcoma of the heart is a very rare malignancy accounting for less than 1% of all primary cardiac tumours. Most of them arise from the pericardium and the right side of the heart and is considered to be highly aggressive with reduced survival rates. Diagnosis in these rare locations is also challenging. We report a 42-year-old gentleman who presented to us with productive cough, chest pain and paroxysmal nocturnal dyspnea. Echocardiography revealed a calcified left atrial mass arising from the posterior leaflet of the mitral valve and radiologically was thought to be a benign atrial myxoma. Excision was planned with histology showing a malignant biphasic spindle cell tumour exhibiting marked cellular atypia and numerous mitoses. On immunohistochemistry, the glandular component expressed diffuse positive staining for Bcl-2 and EMA with focal positive staining for pancytokeratins. The spindle cell component expressed CD99 and EMA and was found to be negative for CD34, S100, desmin, Melan-A and HMB-45. Cytogenetic testing revealed SS18-SSX1/2 gene fusion with SS18 rearrangement confirming the diagnosis of synovial sarcoma in this rare location. A postoperative computed tomography was performed which showed no evidence of metastasis or primary lesions elsewhere. There was excellent postoperative surgical recovery and adjuvant chemotherapy was considered in the multi disciplinary meeting. Primary cardiac synovial sarcoma is an extremely rare malignancy especially when arising from the left atrium posing diagnostic difficulty mimicking atrial myxoma. In contrast to the poor prognosis mentioned in the literatures, there was excellent recovery in this gentleman.

Histological Findings in Swyer-James (MacLeod) Syndrome: Report From Three Adult Cases Managed Surgically With Emphasis on the Vascular Network and Radiological Correlation.

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Swyer-James-MacLeod syndrome (SJMLS) is a rare lung condition that manifests radiologically as unilateral hemithorax lucency as a result of post-infectious obliterative bronchiolitis, leading to small airways obstruction and secondary emphysema. The histological features of SJMLS are poorly and infrequently described. We present three cases of the syndrome that underwent lobectomies in our institution from 2013 to 2015, in three women, aged, 33, 46 and 22 years, presenting with recurrent lower respiratory tract infection, shortness of breath and pleuritic chest pain. Two underwent left upper lobectomies, one left lower lobectomy. The first case demonstrated hyperlucency of the affected lobe with markedly reduced blood vessel attenuation. The radiological findings of the second case were of extensive bronchiectasis, hyperlucency, mucus plugging and hypervascularity. The radiological findings of the third case were of an apical bulla and upper lobe cavitating lesion with lobar hypolucency and hypoperfusion. The main histological findings were bronchiolar changes with bronchiolectasis, mucus plugging, constrictive / obliterative bronchiolitis and various degree of peribronchiolar inflammation. Emphysema was mild and diagnosed as loss of attachment of alveolar walls. In addition, Case 1 had dystrophic, hypoplastic or absent branches of the pulmonary arteries. Case 2 showed prominent bronchial arterioles and abnormal tortuous dilated pulmonary arteries and veins. Case 3 had established bronchiolar scars in the bronchovascular bundles, pleural arteries showed medial hypertrophy and the interlobular septa contained dilated prominent veins, as well as cystically dilated inflamed peripheral bronchus. These cases highlight the importance of vascular changes in SJMLS, likely secondary to the bronchiolar inflammation and destruction leading to capillary bed destruction from secondary emphysema and reactive pulmonary and arterial changes.

Endobronchial Ultrasound (EBUS) is a Highly Accurate and Minimally Invasive Procedure for the Investigation of Mediastinal and Hilar Lymphadenopathy, Both in Malignant and Non-Malignant Conditions.

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Mediastinal nodal staging with EBUS is recommended for patients with resectable non-small cell lung cancer and has emerged as a safe tool to establish granulomatous pathology in suspected sarcoidosis.
Methods: We conducted a retrospective analysis of the outcomes of EBUS performed in a large teaching university hospital with a rapid access lung clinic over a 12 month period and correlation with endobronchial and CT guided biopsies, and surgical resections, when available, and compared the adequacy of EBUS when performed with and without rapid on-site evaluation (ROSE).
Results: In 2014, 161 patients underwent EBUS, 100 males (62.1%) and 61 female (37.9%). Mean age 48.98 years [range 23-89]. A total of 222 lymph nodes were sampled (mean 1.37). ROSE was performed in 80.1%, with a mean of 2.6 MGG slides per lymph node (range 1-12). Cell blocks were prepared in 95% of cases. Immunohistochemistry was performed in 85.5% of malignant cases. Overall inadequacy rate was 9.9%; (8.8 % for sarcoidosis, 15% for malignancy, 11% without ROSE, 5.8% with ROSE).
70.5% of EBUS samples for sarcoidosis show granulomas. Malignancy was diagnosed in 34.2% of cases (pulmonary adenocarcinoma 34.5%, squamous cell carcinoma 26.6%, small cell carcinoma 18.1%, non small cell carcinoma NOS, 7.3%, extrapulmonary malignancies 16.4%). 87.5% of cases with prior history of malignancy or a high degree of clinical concern were diagnosed as malignant. Where available, histological diagnosis concordance was seen in 83.3% of malignant cytology. Molecular studies were attempted on 44.8% of non-small cell pulmonary carcinomas (16 cases); one case demonstrated ALK rearrangement.
Conclusion: EBUS —TBNA of mediastinal lymph nodes has a excellent yield for sarcoidosis and by providing adequate tumour volume, allows for precise subtyping with immunohistochemistry and molecular studies.

Audit of Epidermal Growth Factor Receptor (EGFR) Mutation Analysis in Non-Small Cell Lung Cancer (NSCLC) in an Irish Cohort

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Purpose of Study: Mutational analysis of Epidermal Growth Factor Receptor (EGFR) is now standard practise in selected cases of Non-Small Cell Lung Carcinoma [NSCLC]. An audit was performed to determine the number and histological type of NSCLCs tested with analysis of the frequency and trend of EGFR mutations in our cohort over time, with particular emphasis on comparing the adequacy of histology versus cytology specimens for use in testing.
Methods: Retrospective review of all specimens sent for EGFR mutation analysis in our institution from December 2009 to February 2015. Patient demographics, specimen type, adequacy, EGFR mutation status and mutation type were recorded. Two testing laboratories (both off site) were used, one 2009-Aug 2012 and another Sept 2012-2015.
Summary of Results: 351 specimens from 341 patients were analysed incorporating primary and metastatic lesions. In 13 cases an EGFR result was not available. 29 cases (8.3%) exhibited mutations (13 (44.8%) cytology, 14 (48.3%) histology, 2 modality unknown), occurring in 22 (76%) females and 7 (24%) males. All cases were Adenocarcinomas. Exon 21 (L858R) and Exon 19 (del) mutations were the commonest mutations with 3 patients exhibiting 2 separate EGFR mutations. Mutations were identified in 12 primary tumours (41%), 15 metastasis (52%) and 2 cases were unknown (7%). Up to Aug 2012, mutation rate was 6.5% (153 specimens tested) while thereafter mutation rate was 9.6% (187 specimens tested). 19 (5.4%) sample (s) were insufficient/inadequate for EGFR testing (10 cytology, 9 histology; Fischer's exact test, p=0.1525).
Conclusions: The overall EGFR mutation rate is 8.2%. Cytology Samples are an important source of material for mutational analysis with no significant difference between histological and cytological samples in terms of adequacy.

Cryoprobe Bronchoscopic Transbronchial Biopsies in Interstitial Lung Disease: Experience of an Irish Interstitial Lung Disease Centre

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Background and aims: In Interstitial lung disease (ILD), when an aetiological factor appears absent and clinical-radiological correlation is non-contributory, histology is required. The traditional surgical lung biopsy (SLB) is not without risks. Cryotechnically obtained specimens contain more alveolated lung tissue and less crush artefact than conventional transbronchial biopsies and may offer an alternative to SLB in selected cases. We aimed at studying the complications of cryoprobe transbronchial lung biopsy (CPBx) and the quality and pathological characteristics of the tissue obtained.
Methods: This is a prospective study of patients who were selected for CPBx including cases of possible/probable idiopathic pulmonary fibrosis (IPF). Complications of the procedure as well as the quality and pathological characteristics of the tissue are studied.
Results: Twenty-seven procedures were performed in 24 patients, 20 of which were radiologically IPF. A total of 77 biopsies were obtained (Average 2.85 biopsies per procedure). Only one was inadequate initially. Fibroblast foci and features consistent with usual interstitial pneumonia (UIP) pattern were present in 18 biopsies from 16 patients (66.7% of total; 80% of suspected IPF cases). Granulomas were identified in 4 patients (16.7%), 3 of which were radiologically suspected IPF (15% of suspected IPF cases). Two patients (8.3%) had organizing pneumonia; both were inconsistent with IPF radiologically. The findings in the remaining 3 patients were nonspecific; two of these were radiologically IPF (10% of IPF cases). Seven patients (25.9% of procedures) developed pneumothorax, only 2 of them (7.4%) required chest tube drainage. Five patients (18.5%) developed bleeding (moderate in 3 (11.1%) and mild in 2 (7.4%)).
Conclusion: CPBx was useful in this cohort at potentially identifying features not typical of IPF and displayed an acceptable complication rate.

The Amount of Autophagy-Related Cardiomyocyte Cell Death is Associated with the Type of Pathogenic Mutation in Genetic Dilated Cardiomyopathy

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Introduction: Genetic dilated cardiomyopathy is a heterogenous group of diseases caused by mutations in various genes. Several types of cardiomyocyte cell death have been implicated in dilated cardiomyopathy: (macro)autophagy-related cell death, apoptosis, necroptosis and oncosis. One plausible mechanism of genetic cardiomyopathy is proteotoxicity of accumulated protein aggregates. We investigated the association of such aggregates as sign of autophagy-related cardiomyocyte cell death with specific pathogenic mutations.
Methods: Hearts from 30 patients with a genetic dilated cardiomyopathy or a combined phenotype of dilated and arrhythmogenic cardiomyopathy were included. Microscopic slices from 8 regions were immunohistochemically stained for P62, a marker for aggregated proteins destined for autophagy.
Results: Sporadic P62 positive cells were seen in control hearts (0.5% of cardiomyocytes, range 0.1-0.8%). Troponin mutations (TNNT2 and TNNI3; 0.7%, range 0.2-1.2%, n=3) showed hardly any increase in P62. Titin (1.6%, range 0.7-2.7% ,n=5) and lamin A/C (1.7%, range 1.1-2.5%, n=5) mutations showed a threefold increase in P62 staining. A tenfold positive staining was found in desmosomal mutations (PKP2 and DSP; 3.8%, range 3.7-4.0%, n=3) and myosin mutations (MYH7 and MYBPC3; 4,6% range 3.3-5.7%, n=3). Phospholamban mutations (8.8%, range 4.1-16%, n=8) and desminopathies (desmin and Alpha-B crystallin; 17% of cardiomyocytes, range 4.0-31%, n=3) showed the highest number of P62 positive cells.
Conclusion: Accumulation of P62 positive protein aggregates is associated with the type of mutation underlying the dilated cardiomyopathy. Titin, lamin A/C and troponin mutations revealed little protein aggregation, whereas desminopathies, phospholamban, desmosomal and myosin mutations show abundant aggregates. This suggests that the type of mutation plays an important role in determining distinct mechanisms of cardiomyocyte cell death.

Major Trauma Centre Status and its Impact on the Department of Cellular and Anatomical Pathology in a large Tertiary Referral Centre

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Background: Major Trauma has been centralised into Major Trauma Centres which act as the focus of Major Trauma Networks. In April 2012, Derriford Hospital in Plymouth, Devon became operational as the regional Major Trauma Centre for the South West Peninsula. As a result, there was potential for an increased number of trauma-related deaths to be referred to the local coroner, as well as surgical specimens, potentially increasing the work load on pathologists. The case mix could include post-operative cases, neurosurgical cases, polytrauma cases and forensic cases.
Methods: On admission, all eligible trauma patients are recorded onto the Trauma Audit & Research Network (TARN) database. The TARN data was retrospectively analysed and cross referenced with the Department of Cellular and Anatomical Pathologies database to determine how many patients had died, how many had post-mortem examinations were performed and how many surgical specimens were sent, on patients from outside the region or transferred from smaller Major Trauma Units.
Results: Over the first two years, there was a small increase in workload from patients who, prior to Trauma Centre status would have gone to other centres.
Conclusions: In recieveing patients from elsewhere in the region, there was an increase in workload for both autopsy and non-autopsy work. This excluded some neurosurgical cases, which traditionally would have been referred (as Derriford is the neurosurgical centre). There are several areas for implication including, APT time, mortuary space and non-autopsy surgical work. Although the workload increase is small, at a time when services are being stretched it is important to ensure any increase in work will not be the "straw that broke the camels back" and can be dealt with accordingly.

An Algorithmic Approach to the Postmortem Investigation and Histological Sampling of Potentially Asbestos Related Deaths

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Purpose of Study To attempt to streamline general pathologist's approach to the investigation of potentially asbestos-related deaths
Methods Turnaround times, tissue sampling protocol and frequency with which samples were sent for formal fibre counts was investigated for 100 consecutive coronial autopsies at the author's institution. Colleagues at other institutions were questioned about their own practice when investigating cases of potential asbestosis, lung cancer or mesothelioma.
Results The author found no consensus in opinion on methods of sampling of the lungs in potential asbestosis, lung cancer or mesothelioma.
The most common indication for samples to be sent for asbestos fibre counts was for malignant mesothelioma. Sending tissue for fibre counts led to considerable delays in the authorisation of postmortem reports and to significant cost implications.
The author presents a pragmatic algorithmic guide to approaching potentially asbestosis-related deaths with suggestions for sampling the lungs and tumour in all cases. In general terms, malignant mesothelioma previously confirmed premortem with histology and immunohistochemistry should not require extensive postmortem histological sampling. Lung cancer and asbestosis require widespread sampling of lung tissue to determine amphibole count according to Helsinki criteria in the former, and in the latter, assessment of the distribution and degree of fibrosis in addition to fibre count. One or more of these tissue blocks can be sent for formal counts in equivocal cases after following the algorithmic approach.
Conclusions Although predominantly intended as a pragmatic approach to assist the busy practicing autopsy pathologist, the author believes that the algorithm presented will help departments streamline their approach to these cases and help the relative of the deceased gain access to compensation when appropriate in a more timely fashion.

Focal Lymphocytic Inflammatory Infiltrates in the Myocardium of a Child Found at Post Mortem. Challenge of Diagnosis.

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Purpose of the Study: This is a case report of a three year old girl who died suddenly at home. An autopsy was performed in order to determine the cause of death.
Method: An autopsy was conducted which showed no gross abnormalities. Microscopy of the main organs and microbiological samples were taken for further assessment.
Results: Histological assessment of the heart showed multiple small foci of lymphocytes around vessels and within the interstitum of the epicardium, myocardium and subendocardium. These lymphoid aggregates consisted of 20-30 lymphocytes up to larger numbers of 100 lymphocytes collectively. Several foci were present within virtually all of the sections taken in both right and left ventricles. There was no evidence of myocyte necrosis. Immunohistochemistry confirmed they were of T lymphocyte cell origin admixed with smaller numbers of macrophages.
Histology from the respiratory system showed a diffuse subepithelial lymphocytic infiltrate in the larynx and trachea, and the nasopharyngeal samples detected Coronavirus, Adenovirus and two types of Parainfluenza virus. However, viral polymerase chain reaction (PCR) from the cardiac tissue was negative.
Conclusion: An unequivocal diagnosis of a myocarditis could not be made in this case due to the lack of myocyte necrosis and the absence of viral DNA within the cardiac tissue. Genetic testing was strongly advised as splenic material had been taken at autopsy and following molecular genetic techniques a mutation was detected in the sodium channel indicating an inherited ion channelopathy. Further genetic counselling and testing of the remaining siblings and family members is being performed.

Fatal Haemorrhage From Varicose Veins, A Rare Cause Of Death?

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Varicose veins affect a third of the UK population. Isolated case reports and small series of fatalities resulting from varicose vein haemorrhage appear in the literature infrequently. Some of the earliest reports of fatality we have found appear in British Medical Journal (1958) and the Lancet (1973), more recently they have appeared in journals of forensic pathology. Our purpose is to establish and bring attention to the rarity of fatality resulting from varicose vein haemorrhage and the importance of the scene of death and autopsy findings. A literature review was undertaken, we obtained relevant Office of National Statistics (ONS) mortality data for the years 2011-2013, and reviewed our own post-mortem records for demographic, clinical and scene of death information in cases we have encountered.
Our findings confirm that fatality resulting from varicose veins remains a rare cause of death. Some of these deaths are preventable and in 2013 NICE (National Institute of Health and Care Excellence, UK) issued guidelines in which haemorrhage from varicose veins constitute a vascular emergency. Importantly emphasis on first aid is required, simply elevating the limb stops bleeding and is life saving, whereas direct pressure and tourniquets do not.
Pathologists should be aware of potential findings at autopsy in these cases. In particular, awareness that even obscure minor injury to a varicose vein could have resulted in significant blood loss leading to death. Blood lost at the scene will not be apparent at autopsy, and details of blood loss could be variably recorded on the scene of death information provided, therefore vigilance is required.

Unexpected Opportunistic Infections in an Era of Chronic Immunosuppressive Therapy: A Report of 9 Cases.

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Histopathologists practice in an era of ever advancing medical treatments for a wide variety of oncological, neurological, haematological and rheumatological diseases.
Immune modulating therapies are taking a more prominent place in clinical practice. However, with such great advances in therapy comes great risk, with the potential of life threatening opportunistic infections in our patients. We present a series of 9 immunosuppressed patients who acquired such infections and in whom the diagnoses were made by histopathological examination. The spectrum of these pathogens ranges from viral (CMV, EBV, Herpes), parasitic (strongyloides) to fungal (P. jirovecii, cryptococcus), and the range of infections is diverse. Our series includes 4 males and 5 females, with an age range of 32 - 72 (mean age = 57 years). Unsuspected infectious diagnoses were made at post mortem in 6 of the 9 cases. Organs affected included lung (n = 5), brain (n = 1) and haematological system (n = 1). In one case both colon and lung were affected (n=1) and in a further case both liver and lung were affected (n=1). Immunohistochemistry and/or histochemistry was invaluable in making the diagnoses and was used in all 9 cases (n=9). Treatments leading to immunosuppression included chemotherapeutic agents, monoclonal antibodies, steroids and methotrexate. We believe that with the ever increasing use of immunosuppressive therapies (both new and old) for a wider number of disorders, vigilance should be paid to their potential to cause life threatening side effects. Histopathologists play a pivotal role in the recognition of this risk and in the diagnosis of these diseases.

Audit of Hospital-Based Adult Autopsy Practice in a University Hospital from July 2013–2014

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The contribution of hospital-based autopsy practice to improvements in patient care is substantial; however, there remains a void in the processes of audit and raising quality of standards in autopsy services. We aim to assess the current autopsy practice compared to RCPath guidelines and identify areas for achieving a high quality autopsy service. All adult autopsy cases performed at a university hospital mortuary between July 2013 and 2014 were reviewed. A total of 522 adult autopsies were performed by 5 Consultant Histopathologists. Ninety nine percent were Coroners' cases. The median turnaround time was 38.5 days, with a range of 3-123 days, excluding 31 outlier cases (complex time-consuming cases). There was considerable variation in turnaround times in complex cases and between the various reporting pathologists. Eighty five percent of cases were compliant with RCPath Minimum Dataset for Autopsy Practice. The remainder were lacking clinical information only. Histology and toxicology contributed to cause of death in 34.4% and 14.8% respectively. No organs were retained. Further review of the cases not compliant with RCPath guidelines (15%), identified that the possible reasons were the inaccuracy, and sometimes irrelevance to the cause of death, of the information received by the pathologists. In many instances, the clinical information given to the pathologist may be controversial, and a certain degree of caution needs to be implemented to avoid including misleading information in the autopsy report. The turnaround times could be improved if a preliminary report is issued within a set time frame, to be followed by the complete report when the histology and toxicology results are available. However, this practice is not acceptable to some coroners who prefer one complete final report. Variations in autopsy practice are to be expected as each autopsy involves substantial case-specific information to which a case-specific answer to the cause of death is expected.

Altered Endosome Biogenesis in Prostate Cancer Identifies Potential Diagnostic Biomarkers

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Prostate cancer is the second most common form of cancer in males, and the incidence of this disease is predicted to double globally by 2030. More than 1.1 million new cases of prostate cancer are diagnosed each year and two thirds of these patients are from the Western world. The current PSA-based test for the diagnosis of prostate cancer lacks specificity, results in missed-diagnoses, over-diagnosis and unnecessary biopsies/treatment. There is an urgent need for a method that enables early accurate detection of prostate cancer. Endosomes and lysosomes are cellular compartments that degrade and turnover macromolecules in order to maintain cellular homeostasis. These organelles are directly involved in the critical processes of energy metabolism, cell division, and intracellular signalling, which are all hallmarks of cancer pathogenesis. Endosomes have a critical role in controlling the secretion of proteins into extracellular fluids, making them an ideal system to identify new biomarkers that are released from cancer cells. We have discovered that endosome biogenesis (formation and function of endosomes) is altered in prostate cancer. There were significant changes in the gene and protein expression for 19 endosomal proteins and differential distribution of endosome subsets in prostate cancer cell lines. There were also changes to the endosomal traffic and signalling of the transferrin receptor in prostate cancer cells. These fundamental changes in the cell biology of prostate cancer have allowed us to identify a specific set of endosomal proteins that have diagnostic potential. We are developing ELISA's to quantify these endosomal proteins in patient samples and antibodies for immune histology applications. The objective for this project is to develop an effective method for the early and specific diagnosis of prostate cancer, which is important as this will have a major impact on patient outcome and survival.

MicroRNA Based Molecular Test for Differential Diagnosis of Morphologically Challenging Melanocytic Lesions

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The incidence of malignant melanoma has rapidly increased in recent times and melanoma currently represents the second most common cancer diagnosed in young adults. Diagnosis is based predominantly on histological assessment; however, due to the wide spectrum of morphological characteristics and lack of firm diagnostic criteria, accurate diagnosis can be challenging. Some atypical melanocytic lesions do not display clear-cut morphological features to allow distinction of benign from malignant tumours, making diagnosis and treatment difficult. Among these atypical melanocytic lesions blue nevi, Spitz nevi and dysplastic lesions are common. From histological features alone, it can be difficult to exclude a diagnosis of melanoma and therefore aggressive surgical strategies may be employed in cases were they are unnecessary, highlighting the need for improved diagnostic techniques. Both mRNA and miRNA profiling have been shown to be able to distinguish benign nevi and primary melanoma tumours. Studying miRNA expression levels is an attractive strategy as miRNAs are highly resistant to degradation and can be easily analysed in FFPE samples. We have studied miRNA expression levels in a cohort of benign, blue, Spitz and dysplastic nevi versus primary melanoma tumours and their derived metastases. Expression levels of key melanoma miRNAs, including miRNA 21, miRNA 211, miRNA 205 and miRNA 200c can be used to distinguish between nevi and malignant melanomas. We propose an easy to implement, simple and robust molecular method based on miRNA expression ratio that, in combination with histological assessment, allows diagnosis of difficult to classify atypical melanocytic lesions.

Effective Molecular Screening in Colorecatl Cancer Patients to Identify Families with HNPCC/Lynch Syndrome in South-East Scotland

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Background: Diagnosis of Lynch syndrome (LS) traditionally relies on clinical criteria to guide diagnostic genetic testing. MMR status of the patient's tumour can help detect Lynch syndrome families as well as having other recognised applications for the patient's management including prognostic and predictive significance. As such, the 'Dataset for colorectal cancer histopathology report' recommendations from the Royal college of pathologists were updated in July 2014 to include screening of colorectal cancer patients under the age of 50 and molecular testing for abnormalities in the mismatch repair genes. In South-East of Scotland we introduced molecular testing to identify individuals at risk of LS. To widen our screening in line with revised guidelines set by European experts, we expanded our cohort criteria to include those between the age of 50 and 60.
Methods: Molecular analysis was carried out on 553 individuals: 446 via 'reflex testing' (newly diagnosed colorectal carcinoma ≤60 yrs, or clinical/ pathological features associated with MMR defects, such as pre-menopausal endometrial carcinoma, multiple tumours and medullary-type carcinomas) and 107 via 'request testing' (clinical criteria and referral dependent). 'Molecular-positive' profiles for LS were identified for genetic pre-testing counselling/diagnostic testing.
Results: 41 patients with potential LS were identified, 24 (58.5%) underwent genetic counselling/testing and 13 cases were confirmed LS with germline pathogenic mutations in the MMR genes. Eight of these were identified using reflex testing. **Conclusion:** This is the first UK study to show that screening for LS in patients with colorectal cancer under the age of 60 is effective at identifying families with LS. The testing protocol is in line with the recent recommendations.

Characterising the Oral and Bowel Microbiome as a Prelude to Understanding its Role in Cancer Development Using a Next Generation Sequencing Approach.

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Purpose of Study: The human microbiome is rich and diverse, especially in the oral cavity and gastro-intestinal tract, where it has been shown to be more stable in adults, although various factors such as diet and antibiotics mays influence its composition.
This pilot study aimed to examine and compare the oral and gut microbial composition in four individuals using a culture-independent approach.
Methods: Saliva and faecal samples were collected from volunteers within the same day on two separate occasions. The V4 region of the 16S rRNA gene was amplified in all samples and PCR products sequenced on an Illumina MiSeq. Unique barcodes were used to sequence 24 multiplexed libraries together. The data were analysed using the Quantitative Insights into Microbial Ecology (QIIME) software. A second series of 42 samples of faeces from 3 individuals were run to investigate consistency over time.
Summary of Results: Operational taxonomic units (OTUs) were assessed and showed 5 major phyla represented in the saliva samples: Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria and Actinobacteria. Similar phyla except forFusobacteria, were found in the stool samples. The weighted Unifrac PCoA analysis displayed a clear separation of the 2 sample groups, and also showed a more disperse bacterial profile for the saliva samples, based on population sizes, whereas rarefaction curves and unweighted analysis indicated higher bacterial diversity in the stool samples. Each individual could be distinguished either by oral or faecal microbiome. One volunteer who had had previous radiotherapy to the mouth displayed a particularly distinct oral microbiota.
Conclusion: The microbial community profiles of saliva and faecal samples of four individuals were found to be distinct from each other, despite sharing similar phyla. Analysis of multiple samples from each volunteer clearly separated each sample by volunteer and by sample type.

Are Current Automated Approaches for Determining the Phylogeny of Multiple Deposits Capable of Interpreting the Complexity of Cancer Evolution?

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Tumour heterogeneity is central to chemotherapy resistance and disease progression in advanced malignancy. This heterogeneity arises due to the evolution of clones within the tumour cell population; the advent of high throughput sequencing has allowed the detection of different tumour cell clones within and between primary tumours and their metastases, potentially allowing mapping of tumour evolution. Several, automated bioinformatic approaches have been devised for determining tumour phylogeny from changes in genomic copy number (CN); either by the overall similarity of genomic changes between tumour deposits or by examining the occurrence of shared breakpoints.
We have compared these automated approaches with a manual determination of phylogeny based upon shared breakpoints identified from four cases of metastatic colorectal cancer consisting of between 6 and 53 deposits. We illustrate several recurrent issues identified with the use of automated systems for the determination of tumour phylogeny associated with an inability to correctly identify and interpret changes in ploidy, an inability to identify heterogeneity within tumour deposits, the masking of smaller events by larger ones, over-interpretation of convergent, but unrelated events, over calling sequencing artefacts as changes in CN, and non-calling of genuine CN changes due to low tumour cell content or low sequencing depth. We conclude that manual interpretation of bioinformatics data is still required to determine the phylogeny of metastatic cancer within an individual.

Tubulin alpha 8 (TUBA8) is Down-Regulated in Follicular and Small Lymphocytic Lymphoma

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Background: Tubulin alpha 8 (*TUBA8*) is a member of alpha tubulin family. We observed a unique pattern of TUBA8 immunostaining in lymph nodes (LNs): predominantly in germinal centres with less staining in mantle and inter-follicular zones. We studied the expression of TUBA8 in different lymphomas to investigate its role in lymphoma.
Methods: Immunohistochemistry was performed for TUBA8 using a monoclonal antibody on TMAs (tissue micro-array) of reactive LNs, follicular lymphoma (FL), small lymphocytic lymphoma (SLL), two sets of transformed diffuse large B cell lymphoma (DLBCL) with concurrent or previous FL, de-novo DLBCL germinal centre (GC) phenotype and de-novo DLBCL activated B cell (ABC) phenotype. TMAs were scored semi-quantitatively. Two sample t-tests were used to compare each group with reactive LNs.
Results: Mean scores of FL (4.93) and SLL (4.87) were significantly lower than that of reactive LNs (6.41); p value=0.0000979 and 0.00004.65 respectively. The mean scores of transformed DLBCL [6.67 (previous FL) and 6.04 (concurrent FL)] and de-novo DLBCL [6.47 (ABC) and 6.24 (GC)] were not significantly different from that of reactive LNs. Noteworthy, grouped transformed DLBCLs and grouped de-novo DLBCLs were significantly different from individual FL and SLL groups (all p values ≤ 0.00001).
Conclusion: TUBA8 is down-regulated in FL and SLL compared with reactive LNs. DLBCL of all types show expression of TUBA8 at a level similar to reactive LNs, and significantly higher than FL and SLL. Whether these findings reflect pure proliferation status or if TUBA8 has a driving biological role in lymphoma, remains to be discovered.

Challenging the Royal College of Pathologists' 2000 Cells Sample Size Required for the Accurate Quantification of Ki-67 Proliferative Indexes (PIs) in Gastroenteropancreatic Neuroendocrine Tumours (GP-NETs)

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Background: The Royal College of Pathologists' guideline on reporting GP-NETs states that Ki-67 PIs should be assessed for grading and suggested a sample size of 2000 cells in areas of highest positive staining. There was little supporting evidence for the suggested sample size.
Aim: To compare the accuracy of counting smaller sample sizes for Ki-67 PIs against 2000 cells as recommend by the Royal College of Pathologists.
Methods: Sections from at least 90 GP-NETs were immunostained for Ki-67 and microscopic images were analysed using ImageJ 'cell counter' tool. Ki-67 PIs were recorded at intervals of 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 cells respectively. The 2000 cell PI was considered the 'gold standard' for comparison. Two independent researchers performed the counting.
Results: Levels of agreement between each sample size and the 'gold standard' were evaluated using Bland—Altman plots. 7 separate pairwise comparisons were performed. Some small sample sizes were shown to have small mean difference and narrow limit of agreement. The Ki-67 PIs were then translated into grades and similar comparisons were performed by calculating the kappa score for categorical variables.
Additionally, the interobserver variation between the two independent researchers were calculated.
Conclusion: Smaller sample sizes (below 1000) tend to overestimate the Ki-67 PIs, possibly due to the effect of concentric counting starting from the center of the hotspot. However, the Ki-67 PIs do start to stabilise closer to 2000 (e.g. 1500). The interpretation of whether a lower sample size can replace the current standard would be a subjective decision, but the kappa score gives a rough idea of how much it affects the clinical grading. Updated data will be presented.

Enhanced Regulation of Cell Cycle and Suppression of Osteoblast Differentiation Molecular Signatures by Prostate Cancer Stem-Like Holoclones

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Purpose of the Study: Targeting the stem cell properties of tumor-initiating cells is an avenue through which cancer treatment may be improved. Before this can be achieved, so-called cancer stem cell (CSC) models must be developed and characterized in specific malignancies.
Methods: In this study, holoclone formation assays were used to characterize stem-like molecular signatures for prostate cancer (PCa) cells.
Summary of Results: LNCaP and PC3 parent cells were capable of responding to stem cell differentiation morphogen retinoic acid (RA), suggesting the presence of inherent stem-like properties. LNCaP cells, which represent early, androgen-responsive disease, formed holoclones after twenty six days. PC3 cells, which represent advanced, metastatic, castration-resistant disease, formed holoclones after only six days. Holoclones displayed decreased expression of RA-genes, suggesting a more immature, less differentiated phenotype. Gene and mircoRNA arrays demonstrated that holoclones downregulated a number of stem cell differentiation regulators while displaying enhanced regulation of G2 to M transition and the mitotic spindle checkpoint components of the cell cycle. PC3 holoclones displayed pronounced downregulation of known regulators of osteoblast differentiation from mesenchymal stem cells and Epithelial Mesenchymal Transition.
Conclusion: Our results suggest that some PCa cells retain the ability to transition to a more immature state in which differentiation and metastatic mechanisms are changed. The highlighting of osteoblast differentiation regulators in this mechanism is particularly notable, considering the propensity of PCa to metastasize to bone.

Inhibition of MyD88 Facilitates Primed-State Transition During Differentiation of Nullipotent Embryonal Carcinoma Cancer Stem Cells

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Background: Cancer Stem Cells (CSCs) are highly tumourigenic in the undifferentiated state only. As such, forced differentiation of CSCs is a potential clinical CSC-targeting mechanism. Historically, treatment with Retinoic Acid (RA) has yielded strong force-differentiation results in pre-clinical studies but its use does not translate to the clinic. We have previously described a role for Toll-Like Receptor (TLR) Signaling modulator MyD88 in malignancy pluripotency. The aim of this study was to further elucidate this mechanism with a view towards developing improved methods of forced differentiation.
Approach: MyD88 was inhibited (peptide-inhibitor) in nullipotent embryonal carcinoma (EC) CSC cell line 2102Ep [nullipotent] and resulting cell types isolated and analysed via flow cytometry.
Results: Initial studies indicated that inhibition of MyD88 forced 2102Ep CSCs to transition in to a 'Primed Undifferentiated State' (PUS), which was responsive to differentiation by RA. Further analysis demonstrated that 2102Ep cells contain 2 sub-populations, only one of which is capable of RA-induced differentiation via PUS transition. The first population maintains nullipotency through maintained high expression of pluripotency markers SSEA4, Oct4, Sox2 and Nanog, which are lost during differentiation of the PUS sub-population. This mechanism is highly adaptive: PUS CSCs can return to the nullipotent, differentiation-resistant and highly-tumourigenic state if MyD88 inhibition is removed. Similar mechanisms are likely employed in vivo, which may explain the failure of RA treatment strategies in the clinic.
Conclusion: Our data indicates that MyD88 is a Differentiation-Gate-Keeper in pluripotent malignancy. It is likely that specific Gate-Keepers operate in other malignancies. Combining Gate-Keeper targeting with standard CSC morphogens increases force-differentiation efficiency

Platelet Cloaking of Cancer Cells is Universal and Drives EMT

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Background: Systemic spread of primary carcinoma resulting in metastatic disease is the main cause of death from solid tumours, yet the molecular mechanisms driving metastasis are poorly understood. This study focused on the intermediate cells in the metastatic cascade, CTCs. Previously we showed that platelets, through direct interaction, aid survival and drive the metastatic profile of ovarian cancer cells. Here we sought to build on this work by examining if platelet cloaking of cancer cells is universal, whether previously seen epithelial mesenchymal transition (EMT) changes are a constant result and if these changes are associated with alterations in stem
Methods: We examined by flow cytometetry the interaction in vitro between platelets and 15 human cancer cell lines of different origin and metastatic potential. The EMT profile of cells 24hr post platelet exposure was assessed by morphology and gene expression analysis (RT-PCR).
Results: Here we showed that platelet cloaking of cancer cells is universal, occurring across all 7 tumour types examined. However, it is heterogeneous with adhesion rates varying both across and within tumour types, from 35% (PC3-metastatic prostate cancer) to 83% (SKMES1-metastatic lung cancer). Changes indicative of EMT were seen in all cell lines. However, again they were heterogeneous in nature; with morphology changes akin to EMT observed at varying degrees across the cancer types. Also, there was no consistent pattern to the EMT-like gene expression changes seen, with one exception a significant increase in the expression of plasminogen activator inhibitor 1 (PAI-1)was observed in 93% of the cell lines examined.
Conclusion: In this study we describe the universal nature of platelet cloaking and that even though the interaction is not inducing precisely the same molecular changes in all the cancer cells; overall it is driving these cells into a mesenchymal phenotype.

Comparative Assessment of HPV Detection Assays in the Management of Women Referred to Colposcopy with Minor Abnormalities

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Background: Cytology based colposcopy referrals include a substantial number of women with minor abnormalities, only a small proportion of which are at risk of developing high-grade disease. Appropriate management strategies should therefore be investigated. In this study, we evaluated the role of HPV DNA testing and mRNA testing in the management of women presenting in colposcopy with minor abnormalities.
Methods: Study participants were recruited at the Colposcopy clinic in the Coombe Womens & Infants University Hospital following an abnormal cytology referral. At the clinic, a smear sample was taken for cytological evaluation and the residual sample was processed for HPV testing with the Cobas HPV DNA test and the Aptima HPV mRNA assay. Clinical performance of the assays was evaluated in comparison with histological diagnosis.
Results: Clinical performance of the Cobas HPV test and the Aptima assay was evaluated in women referred with minor abnormalities (n=281). Clinical sensitivity and specificity for detection of CIN2+ was 87.8 % and 56.0% for the Cobas test and 87.8 % and 60.4% for the Aptima. Analysis was also stratified by referral cytology and the sensitivity and specificity of the Cobas test in the LSIL category was 88.1% and 48.5% versus 91.5% and 51.4% for the Aptima assay. In the ASCUS category, the sensitivity and specificity of the Cobas test was 87.5% and 65.8%. The Aptima test displayed slightly lower sensitivity at 82.5% but much higher specificity at 72.1%.
Conclusion: Both tests performed comparably, however the Aptima test has better specificity in correctly identifying women at low risk in the ASCUS referral cytology.

Evaluation of Triage Strategies for Management of HPV DNA Positive Women Presenting at Colposcopy

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Background: HPV DNA testing has improved the management of cervical disease. However due to the high prevalence of transient HPV infections, specificity of HPV testing is limited. There is a clear need to improve the specificity of HPV DNA testing in order to avoid un-necessary testing and follow up. In this study we evaluated alternative approaches; HPV 16/18 genotyping, HPV mRNA and p16/Ki67 expression to triage HPV DNA positive cases presenting in colposcopy.
Methods: Patients were recruited at the Colposcopy clinic in the Coombe Womens & Infants University Hospital. A smear sample was taken for cytological evaluation and the residual sample was tested with the Cobas HPV DNA test, Aptima mRNA test and CINtec (p16/Ki67).Clinical performance of the assays was evaluated in comparison with histological diagnosis.
Results: 875 women were tested in total. 57% (310/542) of women with minor abnormalities were HPV positive of which 40.3% had HPV 16/18 genotype and 88% were mRNA positive. 85.2% (284/333) of women with high grade disease were HPV positive of which 60.5 % had HPV 16/18 genotype and 96.1% were mRNA positive. The Aptima test had higher sensitivity to predict CIN2+ at 97.1% compared to HPV16/18 genotyping at 61.7%. The specificity of the Aptima assay was 20% versus HPV16/18 genotyping at 57.2%. 100 patients had all three tests performed. Positivity for Cobas, Aptima and CINtec was 82.3 %, 70.5% and 52.9% in <="" p="">">

Development of Diagnostic Test for *H3F3A* p.Gly34Try (G34W) Mutation in Giant Cell Tumours of Bone Based on Droplet Digital PCR

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Giant cell tumours of bone (GCT) are primary locally aggressive bone tumours with a recurrence rate of up to ~30%. The tumour is characterised by numerous osteoclasts and neoplastic stromal cells. Making a diagnosis can be challenging because the differential diagnosis incudes an array of benign osteoclast-rich tumours but also osteoclast-rich osteosarcoma. Recently the occurrence of *H3F3A* p.Gly34Try (G34W) and G34L mutations was reported in 96% of GCT, the latter occurring rarely. These mutations occur in less than 2% of >400 other benign and malignant bone tumours. It has been emphasised that a diagnosis of GCT should be made with caution in the absence of detection of G34W substitution. Given the diagnostic importance of G34W mutation in GCT, we have developed a simple, quick and cost-efficient diagnostic test to detect this recurrent alteration in FFPE DNA using droplet digital PCR (ddPCR). The ddPCR data from DNA of >100 GCT have been compared with previous 'genotype' data generated using Sanger sequencing, and a number of next generation sequencing approaches (whole exome, whole genome and targeted panels). The G34W and G34L can both be detected in a single assay. We have demonstrated the sensitivity, specificity, repeatability and robustness of the test to be very high with a turnaround time of no more than 5 working days. As ~30% of GCT recur locally following curettage a blood test would be valuable to monitor patients. To this end the ddPCR test also shows that the mutation can be detected in plasma.

IDH1 and IDH2 Digital PCR Assay Development for Chondrosarcoma Tissue and Plasma Analysis

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Conventional chondrosarcoma is the most common bone sarcoma in adults with an incidence of ~0.2/100,000/year. Clinical outcome has not changed over 30 years with only ~50% of patients with high grade disease (Grade (G) II, III) surviving 5 years. Patients with a dedifferentiated central chondrosarcoma, a rare variant, derived from conventional CS have a 5% 5 year survival. Typically resistant to chemotherapy and radiotherapy, high grade disease has been treated by surgery for more than 50 years. It has recently been shown that IDH1 and IDH2 mutations are present ab initio in ~60% of chondrosarcoma cases and that these are retained throughout disease progression. This has opened up a number of new potential diagnostic, biomarker and therapeutic options. Digital PCR is currently the most sensitive and accurate method for detecting and quantifying mutant DNA molecules. The BioRad QX200 digital PCR platform is also both cost effective and scalable. Using the QX200 platform, we have developed assays for the 5 common IDH1 mutations and the 1 common IDH2 mutation. We have developed the IDH1 assays both in singleplex and multiplex. We have optimised and validated all assays in tissue samples demonstrating both high sensitivity and specificity when compared to previously genotypes samples. We have demonstrated that the assays are quantitative over 4 orders of magnitude and in high quality DNA we can detect IDH mutations at below 1 mutant molecule in 10,000 wild type molecules. In a pilot study, we have used digital PCR to analyse circulating tumour DNA levels in plasma taken pre-surgery from 14 patients whose chondrosarcoma harbour an IDH1 mutation. It was possible to detect IDH1 mutant molecules in plasma of all Grade III samples, 50% of Grade II and none of the Grade I samples. In 4 of these cases where the ctDNA was also measured post-operatively, the levels of ctDNA dropped dramatically.

Tumour Necrosis Factor Receptor, CD40, Gene Functions as an Oncogene and Promotes Cell Proliferation in Colorectal Cancer Cell Lines

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Introduction: CD40 is a tumour necrosis factor (TNF) receptor which regulates a range of cellular responses. CD40 is activated by its ligand CD40L and may promote tumourigenesis in haematological cancers. However, CD40 functions as a tumour suppressor in solid cancers. CD40 maps to chromosome 20q13, a region which is amplified in 40-50% of colorectal cancer (CRC). The functional activities of CD40 were tested in CRC cell lines for cell proliferation and motility.
Methods: Expression of CD40 was screened in CRC cell lines by western blot. To define the role of CD40 in human CRC, we knocked down CD40 using small interfering RNA (siRNA) and the knockdown was confirmed by qPCR and western blot. The PrestoBlue assay was used to study proliferation in colorectal cell lines, and flow cytometry to study the cell cycle. Transwell migration and wound healing assays were performed to investigate the effect of CD40 on cell motility in CRC.
Result: CD40 was expressed in CRC cell lines HCT116, RKO, DLD1 and HT29, and not expressed in SW480 and SW620 cell lines. Knockdown of CD40 reduced cellular proliferation in HCT116 ($p=0.0158$) and DLD1 ($p=0.0020$) cell lines. Knockdown of CD40 showed a higher number of cells in the sub G0 phase (dead cells) in the cell cycle analysis compared to the control. However, knockdown of CD40 in HCT116 did not have an effect on cell motility in both the transwell migration (HCT116= $p=0.253$) and wound healing assays.
Discussion: CD40 exhibited oncogenic activity in CRC cell lines. CD40 enhanced cell proliferation but not cell motility in CRC cell lines.

CD45 Positive Small Cell Carcinoma and CD45 Negative High Grade B-Cell Non-Hodgkin Lymphoma

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The expression of CD45 (Common Leucocyte Antigen) and cytokeratin is thought to be mutually exclusive with CD45 expression largely restricted to haematological malignancies and cytokeratin expression largely restricted to carcinomas. We report two clinically relevant cases. The first case is a urinary bladder biopsy showing a high grade malignant tumour with cells that had scanty cytoplasm, hyperchromatic stippled nuclei and high mitotic activity. Nuclear molding was present. The tumour cells showed focal strong positivity for CK7 and diffuse positivity for CD56 and synaptophysin. Focal positivity for CD45 was present and confirmed on repeat staining. The morphology and immunoprofile was consistent with a small cell carcinoma showing aberrant CD45 expression. The second case is a maxillary tumour biopsy composed of medium/large atypical lymphoid cells with hyperchromatic nuclei, small nucleoli and scanty cytoplasm. Mitoses and apoptotic cells were noted. Immunohistochemistry showed the atypical cells to express CD20, CD79a, Bcl6 and MUM1 but not CD45, CD5, CD10, cyclin D1, CD30, TdT, ALK1, CD2, CD3, neuroendocrine or melanocytic markers. A high Ki67 proliferation fraction was present. The appearances were consistent with a diffuse large B-cell lymphoma. The above cases highlight the possibility of aberrant expression as well as loss of expression of immunohistochemical markers by neoplastic cells in undifferentiated malignancies. Attention to tumour morphology may provide diagnostic clues. Interpretation of immunohistochemistry in the context of tumour morphology as well as awareness of aberrant expression/loss of expression can help avoid diagnostic error.

A Case of Two Distinct Lesions Within a Lymph Node of a Patient with Invasive Breast Carcinoma

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Accurate, timely diagnosis is the ultimate aim in surgical pathology. Numerous histological pitfalls and lesional mimics exist, with the need to maintain an awareness of such entities vital if potentially serious misdiagnoses are to be avoided. This case report describes two distinct lesions within the same lymph node, both of which are potential mimics of each other. A 43 year old female presented with a three week history of a left breast lump. She had no known previous breast disease or any associated risk factors. A needle core biopsy of this clinically and radiologically suspicious mass yielded a diagnosis of grade 2 invasive lobular carcinoma. Left axillary sentinel node biopsy was thus undertaken. Two hot and blue sentinel lymph nodes were excised. One was free of neoplasia, the second contained benign naevus cell inclusions within the capsule together with a micrometastasis. Immunocytochemistry confirmed the presence of two distinct cell populations; the benign naevus inclusion cells stained positively for S100 but not for AE1/3, the reverse pattern was observed in the invasive lobular carcinoma cells. Heterotrophic benign inclusions within lymph nodes are an infrequent yet well recognised entity. Ridolfi et al reviewed the lymph nodes from 909 axillary surgery patients and found 0.017% of lymph nodes contained benign naevus cell inclusions. Small benign naevus cells within the capsule of a lymph node can resemble the 'Indian file' pattern of classic invasive lobular carcinoma. This case is unusual in that both metastatic carcinoma and benign naevus inclusion cells were present within the same lymph node, enabling a clear comparison of the cytomorphology and immunoprofile of these two distinct lesions. An awareness of benign inclusions within lymph nodes helps to avoid the potential for misdiagnosis. The judicious use of immunocytochemistry can be useful in distinguishing benign inclusions from carcinoma.

Basal Phenotype Breast Cancer — A Random Designation in Current Practise?

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Recently there has been increasing recognition of distinct breast cancer phenotypes. Of these, Basal phenotype breast cancer (BBC) has attracted particular interest since the majority are triple negative (TN); have an aggressive natural history and can be associated with BRCA1 germline mutation. This area is mired in difficulty, as a precise unifying definition of BBC remains elusive. Several morphological features more prevalent in BBC have been identified. In our practise we noticed variable use of 'basal' in reports. Given this, whilst no specific therapies to BBC currently exist, we felt it necessary to understand how accurate our designations have been and whether this is a worthwhile practise.
Method: The diagnostic database was searched for all malignant TN breast resections or reports containing the word 'basal' within 12-months. TN was defined as Allred score ER 0-2/8, PR 0-2/8 and HER2 0, 1+ or 2+ negative on FISH. For completeness, we considered including all breast cancers, but pragmatically this was not possible. We carried out CK5 and CK14 staining on all cases where not performed.
Results: Of the 618 invasive breast cancers, 69 cases (11%) were identified, of which 88% were TN and 28% were designated BBC in the report. 16% were both TN and BBC. Where a diagnosis of BBC was made, 37% of cases had additional markers requested. Preliminary results showed 94% were CK5+ and CK14+. This is higher than other studies, implying specificity but not sensitivity in suspecting BBC amongst reporting pathologists.
Discussion: A limitation of this review is that it cannot identify the rare non-TN BBC not diagnosed as such. It also represents current practise in a single institute and may not reflect national practise. We identified patchy use of the designation BBC, with overall under-reporting of this subtype. We recommend that if it becomes necessary to distinguish BBC lesions, additional markers studies, such as CK5 and 14 be consistently performed.

Topoisomerase Iialpha Predicts Survival in Breast Cancer Treated with Neoadjuvant Anthracyclin Based Chemotherapy

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Purpose: Neoadjuvant chemotherapy (NACT) is increasingly used for the management of large but operable, inflammatory, and locally advanced breast cancer (LABC). Little is known about predictors of response/survival following NACT. The topoisomerase IIα (TOP2A) gene, a key regulator of DNA repair and modelling, is thought to be target for anthracyclin and other chemotherapeutic agents. The aim of this study is to assess the role of TOP2A as marker for response/resistance to NACT and patient outcome.
Methods: Patients who underwent NACT, predominantly anthracyclin, for primary and operable invasive carcinoma or LABC in the period between 2005 to 20013 at a single large tertiary referral breast unit were identified. Comprehensive data on chemotherapy regimen, surgical treatment, pathological response and survival were collected. Pre-treatment tumour samples were stained for standard predictive and prognostic markers and TOP2A. Results were correlated with pathological response (PR) and patient survival.
Results: 252 patients fulfilled inclusion criteria. Mean age was 48.94ys. Complete PR was achieved in 15.4%. The mean expression level of TOP2A in pre-treatment core biopsies was 75.4%, range 0-95%. There was significantly higher expression in high grade tumours ($p=0.04$) and positive correlation with ki67 expression ($r=0.405$, $p<0.001$). There was no correlation with nodal status, PR or HER2 expression. Cases with high expression (>50%), had significantly worse overall survival (mean 38 vs 52 months, $p=0.01$). This was also identified in the endocrine non responsive group (ER Allred score≤4), mean 74 vs 33 months, $p=0.04$. On multivariate analysis, TOP2A was not an independent factor for overall survival.
Conclusions: TOP2A protein is expressed in high grade breast carcinoma with high Ki67 proliferation index. Its expression in pre-treatment biopsies predicted patient outcome in the neoadjuvant setting. This strong adverse effect on survival warrents further prospective investigation as a marker of outcome in NACT patients.

Breast Cancer Sentinel Lymph Node Diagnosis by Intraoperative Touch Imprint Cytology, a Reaudit of Results to Inform a Multi-Disciplinary Team Evaluation of the Need for New Technologies

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Purpose of Study: Sentinel lymph node sampling is performed in early stage breast cancer, as per NICE guidance, and determines whether axillary clearance is indicated. At Northumbria Healthcare NHS Foundation Trust we use intraoperative touch imprint cytology (TIC) and provide this service across three operating sites (a round trip of 85 miles). It is crucial that sentinel node evaluation is specific to avoid patients undergoing unnecessary axillary surgery. The audit purpose is to measure the quality of TIC against local and published outcomes, and inform an evaluation of the current service versus potential new methodologies for sentinel node sampling.
Methods: Cases included were those coded as sentinel lymph node procedures over a twelve month period. The number of patients undergoing TIC was recorded. The results were compared to the subsequent histology. The sensitivity and specificity of TIC were calculated.
Summary of Results: 123 patients underwent TIC. In seven the result was positive for malignancy and they went on to have immediate axillary clearance. All seven cases correlated with histology positive for macrometastases (specificity 100%, in line with our previous audit and with published figures). 113 TIC cases were reported as negative. Seven of these had macrometastases on histology (giving sensitivity of TIC 50%, in line with published data).
Conclusions: TIC is a procedure with high specificity and this is crucial to avoid unnecessary axillary surgery. Sensitivity, at 50%, is comparable with published figures and previous local audit. New technologies such as PCR may increase sensitivity but costs of implementation at multiple geographically dispersed operating theatres are high.

Her-2 Status on Primary Breast Cancer and Concurrent Lymph Node Metastasis

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Background: Breast cancer is a heterogenous group of neoplasms. Over expression of HER2/neu gene (HER-2) is an important parameter both in terms of prognosis as well as prediction of treatment to anti-HER-2 treatment. Conventionally, HER-2 over-expression is tested on the primary breast tumour and concurrent lymph node metastasis is not usually tested. This study was carried to know if HER-2 status of concurrent lymph node metastasis is similar or different to that of the primary tumour.
Design: 100 consecutive cases of primary breast carcinomas with corresponding lymph node metastases of greater than 2mm were identified. These cases were randomised and anonymised. Immunohistochemistry was performed using an Oracle kit® . The slides were individually scored by 2 independent assessors. All disagreed results were discussed. All slides that scored a 2+ had reflex FISH testing.
Results: In our study, the concordance rate was 87%. Of the 9% that resulted in primary tumour negative and lymph node positive, eight of these cases could not be explained. 4% that resulted in primary tumour positive and lymph node negative, two of these had received neo-adjuvant therapy therefore potentially altering the tumour characteristics.
Conclusion: 30 cases (31%) were HER-2 positive, either primary tumour positive, lymph node positive or both. Testing the primary tumour alone would determine the positivity in 21 cases (21%). However, testing the lymph node would help us determine HER-2 positivity in 26 % of the cases, a difference of 5% . Testing Her-2 status on concurrent lymph node metastasis should also be considered in breast cancer cases.

Reverse Phase Protein Array is a Useful High Throughput Technique for Assessment of Multiple Proteins in Breast Cancer

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Purpose of study: Immunohistochemistry is a well-established and reliable method for assessment of protein expression in breast cancer (BC). However, simultaneous assessment of several proteins is laborious and time-consuming. High-throughput proteomic techniques such as RPPA can provide a quantitative objective alternative with no need for virtual assessment of protein expression.
Methods: A panel (n=16) of biomarkers (hormone receptors, ER-related proteins, HER family proteins and genes related to apoptosis, proliferation and epithelial mesenchymal transition proteins) has been assessed in reverse phase protein array (RPPA). RPPA was applied to 6 BC cell lines representing different molecular classes corresponding to those identified in human BC tissue (Sorlie et al. Proc Natl Acad Sci 2001,98:10869-10874).
Summary of Results: RPPA successfully produced quantitative assessment of several proteins. The differential expression of these proteins in the different BC cell lines was highly comparable to those reported in cell lines and BC molecular classes using other well-established techniques.
Conclusions: RPPA is reliable and useful high through proteomic technique for assessment of large panel of proteins in BC.

Successful Alignment of Macrodissected FFPE Tissue to Nottingham Prognostic Index Plus (NPI+) Classes Using RPPA Technology

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Background: The NPI+ is dependent on the determination of breast tumour biological class by assessing the expression levels of 10 biomarkers using immunohistochemistry (IHC). However, it currently relies on semi-quantitative assessment and does not reflect subtle changes in protein expression. Reverse Phase Protein Microarray (RRPA) can accurately quantitate protein expression and substitute the subjectivity of IHC.
Aim: To validate the use of RPPA in improving molecular classification of breast cancer in order to stratify the patients according to the NPI+ decision making tool.
Methodology: Total protein was extracted using commercial Q-proteome Qiagen tissue kit from 25 cases macrodissected FFPE breast tumour tissues. Samples were robotically spotted onto nitrocellulose-coated glass slide (MicroGridII). Antibodies for ER, PgR, CK5/6, CK7/8, EGFR, HER2, HER3, HER4, p53 and Mucin 1 were used to quantify protein expression along with house-keeping proteins which were compared with IHC.
Results: Enrichment of tumour using macrodissection provided successful correlation to IHC for 8/10 NPI+ markers except HER3 and P53. Classification of tumours into NPI+ Biological Classes using RRPA showed identical classification as IHC.
Conclusion: RRPA is a useful technique for quantification of proteins in FFPE breast tissue enriched for tumour cells by macrodissection and is comparable to IHC.

Improvement of Ki67 Staining in Breast Cancer (BC)

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Introduction: Inclusion of Ki67 into pathology practice improves the performance of prognostication and prediction model of BC. However, the use of Ki67 as part of the daily implemented prognostic indices in diagnostic settings is still controversial. Few reports in literature demonstrated cytoplasmic and membranous expression of a nuclear Ki67 protein in BC which may affect the scoring and cut-off point reproducibility.
Aim: In this study we have evaluation the cytoplasmic/membranous expression of Ki67 using different optimization conditions.
Methodology: Full face sections were stained using different secondary detection kits, different antigen retrieval times, different pH, different protein blocking, different primary antibody (MIB-1) concentration and different primary antibody clones. Additionally, manually and full-automated IHC staining system were used.
Results: There were no obvious differences that could be appreciated regarding cytoplasmic/membranous staining when different antigen retrieval times, different pH, different protein blocking or different antibody (MIB-1) concentration were applied. Furthermore, there was no change in the cytoplasmic/membranous staining in different secondary detection kits when MIB-1 clone was used. Additionally, the cytoplasmic and membranous staining occurred when using the MIB-1 clone to stain the same cases in the Ventana autostainer with different condition. However, use of a different clone (30-9) with the Ventana autostainer resulted in strong nuclear staining with obvious negative cytoplasmic and membranous reactivity.
Conclusion: Using (MIB-1) anti-Ki67 clone with different optimization conditions is associated with case-specific cytoplasmic/membranous reactivity. In this context, it could be suggested that to utilize different anti-Ki67 clones in order to reach consistency in scoring and subsequently cut-off point.

Role of Mitotic Checkpoint Proteins (BUB Family) in Breast Cancer

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Background: The budding uninhibited by benzimidazoles (BUB) gene family encodes protein components of the mitotic spindle checkpoint machinery. Mutations in BUBs may disrupt the checkpoint and cause chromosomal instability, a hallmark of solid tumours including breast cancer (BC). BUBs are among serine-threonine kinases known to help identify luminal BCs with poorer prognosis [Finetti et al, Cancer Res 2008; 68(3)].This study investigated the role of this kinase family at the protein level, especially within low grade BCs.
Methods: BC tissue microarrays (n=1117) were immuno-stained for BUBs (BUB1, BUB1B & BUB3) and expression patterns correlated with clinico-pathological/molecular variables as well as patient outcome. Computational platforms explored the role of the proteins further in relation to BC grade.
Results: Cytoplasmic BUB1 and nuclear BUB3 revealed positive correlations with luminal-enriched proteins like ER (p=0.012), PR (p<0.001) whereas cytoplasmic BUB1B was negatively correlated (p=0.002). BUB1 and BUB3 were negatively associated with grade and NPI, whereas BUB1B was positively associated. Negative associations were observed between BUB1, BUB3 and proliferation markers, Ki67, p16 and PI3K (p=0.04). Positive correlations were observed with STAT3 (p=0.003) including ER positive (p=0.010) subgroups. Survival analysis revealed associations between BUB1 and long term (15 years) breast cancer specific survival in the whole series (p=0.025) as well as ER positive (p=0.024) subgroups. Computational analysis confirmed BUB3 as predictive of grade in BC (p=0.025) with no added value from Ki67 inclusion.
Conclusions: BUB1 and BUB3 are the key kinases for low-grade ER positive BCs and probably maintain genomic stability, whereas BUB1B is preferentially expressed in high grade disease. Functional studies will further delineate the role of these molecules in low grade BCs. *Project supported by CDF from PathSoc and NIHR

Nottingham Prognostic Index Plus (NPI+): Validation of the Modern Clinical Decision Making Tool in Breast Cancer in an Independent Series

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The Nottingham Prognostic Index Plus (NPI+) is a modern clinical decision making tool in breast cancer (BC) aiming to provide improved patient outcome stratification superior to the traditional NPI. This study aimed to validate the NPI+ in an independent series of BC. 885 primary early-stage BC cases were immunohistochemically assessed for 10 biomarkers [Cytokeratin (Ck)5/6, Ck18, EGFR, Estrogen Receptor (ER), Progesterone Receptor (PgR), HER2, HER3, HER4, Mucin 1 and p53] and classified into biological classes. Subsequently, NPI+ Prognostic Groups (PGs) were assigned for each class using standard prognostic variables. PGs were compared between the Validation and Development series and their role in patient outcome prediction. There was a comparable distribution of biological classes between the Validation and Development (n=1,073) and series. PGs were comparable in predicting patient outcome between series in Luminal A, Basal p53 altered, HER2+/ER+ tumours. The good PGs were similarly validated in Luminal B, Basal p53 normal, HER2+/ER- tumours and the poor PG in the Luminal N class. Due to small patient numbers assigned in the remaining PGs, Luminal N, Luminal B, Basal p53 normal and HER2+/ER- classes could not be validated. This study shows the distribution of the NPI+ Biological Classes is similar in an independent series of primary BC and can conclude that biological class determination using the NPI+ biomarker methodology is similar between patient series. We observed similar patterns of patient outcome in the majority of NPI+ PGs between the Development and Validation series and can conclude that NPI+ prognostic classification for these groups appears robust. Three of the poor PGs were under-represented in the Validation series due to a lower frequency of higher grade tumours and could not be validated in this study.

MED7 in Breast Cancer: Relationship to Grade and Lymphovascular Invasion

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Hypothesis: Mediator complex (MED) proteins, components of the transcription apparatus, have a key role in the transcriptional regulation of genes involved in cancer, including the regulation of ERα in breast cancer (BC). Thus the role of the member MED7 was deemed of interest, especially in low grade ER positive BCs. Differential expression analysis between BCs with positive versus negative lymphovascular invasion (LVI) status on the Nottingham subset of the METABRIC series showed that MED7 was negatively correlated with LVI (p=0.005). Thus its protein expression was also investigated in relationship to LVI, alongside other prognostic parameters. **Methods:** Breast cancer tissue microarrays (n=1260) were immuno-stained for MED7 and expression patterns were correlated with clinico-pathological and molecular variables as well as patient outcome.
Results: Positive nuclear MED7 expression was significantly correlated with positive ER (p=0.001) and PR expression (p<0.001) and was preferentially positive in lobular carcinomas. MED7 expression was associated with tumours of a low grade (p<0.001), small size (p<0.001), good NPI (p<0.001) and negative HER2 expression (p=0.043). A significant negative association was found with LVI (p=0.025) and proliferation markers like Ki67 (p = 0.001). Kaplan Meier survival analysis revealed significant associations with long term breast cancer specific survival (BCSS) even after 15 in the whole BC series as well as ER positive subgroups (p<0.001).
Conclusions: MED7 was significantly associated with low-grade, ER positive tumours and with negative LVI status. This explains its association with increased long-term BCSS and suggests it as a predictor of favourable prognosis.
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Phenotyping Characterisation of Breast Cancers: the Role of CDC Proteins (2, 42 & 42BPB)

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Background: The molecular profile of low grade (LG) luminal breast cancers (BCs) remains unresolved. Usually of good prognosis, a small subset will progress. CDC (cell division control) family genes are among serine-threonine kinases known to help identify luminal BCs with poorer prognosis [Finetti et al, Cancer Res 2008; 68(3)]. In other studies, CDC42 signalling pathways seem to be active in luminal BCs [METABRIC: Curtis et al, Nature. 2012; 486]. This study investigated the CDC kinase family members at the protein level for correlations with morphology, grade, phenotype and prognosis.
Method: BCs (n=1048) of a well annotated series of tissue microarrays were immuno-stained for CDC proteins [2, 42 and 42BPB (binding proteinB) and their expression correlated with clinico-pathological parameters and outcome. Computational platforms explored the role of the proteins in relation to BC grade.
Results: CDC2 and CDC42BPB showed positive association with grade (p=0.015) while CDC42 showed negative association (p=0.009). CDC2 and CDC4242BPB showed negative correlation with ER but positive correlations with HER and basal markers (p=0.001). In contrast, CDC42 expression was positively correlated with PR (p=.044) but negatively with HER2 and basal markers (p=0.03). CDC42 was associated with the tubular and lobular morphology within the low grade BCs (p=0.02) and was associated with longer overall survival (p=0.02) in the whole cohort as well as ER+ (p=0.03) subgroups. Computational analysis confirmed CDC2 as predictive of higher grade in BC (p=0.007) with no added value from Ki67 inclusion.
Conclusion: CDC42 is a key kinase for low-grade BC showing correlations with morphology and receptor status, while CDC2 and CDC42BPB are preferentially expressed in higher grades. Associations of CDC42 to BC morphology probably stem from its role in cytoskeletal remodelling.
*Project supported by CDF from PathSoc and NIHR

The Prognostic Significance of STAT3 Expression in Invasive Breast Cancer: an Immunohistochemical and Reverse Phase Protein Array Study

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Purpose: Signal transducer and activator of transcription-3 (STAT3) is a STAT family member involved in cellular biological functions. However, reports regarding the prognostic impact of STAT3 expression in breast cancer (BC) were variable whether being a factor of poor prognostic or good prognostic impact in BC.
Methods: Immunohistochemical (IHC) expression of phosphor-STAT3 (pSTAT3) was studied in large series of invasive BC (n=1270). pSTAT3 and STAT3 were quantified using Reverse phase protein array (RPPA) on proteins extracted from macro-dissected FFPE tissues representative of 49 cases. STAT3 transcript data from previously generated gene expression data of 128 frozen BC samples were also analysed.
Results: pSTAT3 was expressed in the nuclei and cytoplasm of invasive BC cells. pSTAT3N+ over-expression was positively associated with good prognostic criteria including small tumour size, low grade, good NPI, negative lymphovascular invasion (LVI), positive ER+, PgR+, negative p53, HER2 negative, and low Ki67LI. However, pSTAT3C+ showed significant positive association only with HER2 status. Nucleo-cytoplasmic combinatorial groups were significantly associated with grade, size, NPI, LVI, ER/PgR status, HER2, P53, Ki67LI, and BC molecular classes. Conclusions: Only pSTAT3N+ was significantly associated with improved breast cancer specific survival (BCSS), independently of other factors. pSTAT3 combinatorial phenotypes were significantly associated with BCSS. On RPPA, the mean pSTAT3 and STAT3 expressions were higher in ER+, PgR+, and tumours smaller size. Lower STAT3 gene transcripts were observed in samples from invasive BC cases which had developed distant recurrence. These associations did not reach statistical significance.
The results of this study suggest pSTAT3 nuclear localisation, regardless of the cytoplasmic expression, to be a marker of favourable prognosis. STAT3, therefore, could have context-dependant molecular roles of STAT3 in BC progression.

Heterogeneity of Ki67 Expression in Different Quadrants and Matched Axillary Nodal Metastases of Invasive Breast Cancer

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Purpose of the Study: Cellular proliferation could be reliably assessed through the expression of Ki67. However, intratumoural heterogeneity (ITH) of expression is amongst the several technical issues beyond the lag of its inclusion into the routine BC practice.
Methods: We studied the IHC expression of MIB1 anti-Ki67 antibody in a subset of invasive (n=55) BC using 3-4 full face (FF) tissue sections from different primary tumour quadrants and the matched axillary metastasis in lymph node (LN) positive cases. Assessment was made following the highest expression (HE, hot-spot), lowest expression (LE), and overall/ average expression (AE) in each FF section. Co-efficient of variation, Bland-Altman plots of differences, and Spearman rank correlation co-efficient were used to assess the ITH of Ki67 expression within the same section, between different sections, and between the primary tumour and LN metastases.
Results: Ki67 expression within the invasive tumour was highly variable with more variability in cases of mixed histologic types displayed the highest spatial ITH compared to those of pure special types. Variability of Ki67 HS, AS, and LS within the same FF section ranged from 13-96%. Moreover, variation between the HS, AS, and LS in the studied sections was 2-88%, 0-87%, and 0-96%, respectively. Ki67 expression was perfectly correlated ($r=0.98$, $p < 0.001$) with the highest Ki67 expression in the studied FF sections from the primary tumour.
Conclusions: The spatial heterogeneity of Ki67 expression in invasive BC was evident within a single as well as multiple FF sections from the primary tumour. Using the HS/hot spot scoring in FF is more representative of the growth fraction rather than the AS recommended by the Ki67 in BC working group. The high Ki67 within LN metastasis corresponded to highest primary tumour expression reflecting the temporal HT through clonal expansion.

The 2013 American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for HER2 Testing in Breast Cancer increases the requirement for Reflex In Situ Hybridisation Testing

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Purpose: Accurate determination of tumour human epidermal growth factor receptor type 2 (HER2) status is critical for optimal treatment of breast cancer. In October 2013, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) issued joint updated guideline recommendations for HER2 testing in breast cancer, with a revised algorithm for interpretation of immunohistochemistry (IHC) and in-situ hybridisation (ISH) results. This study investigates the impact on HER2 IHC categorisation, implication for reflex ISH testing and potential for identification of false negative IHC.
Methods: HER2 IHC preparations on 251 invasive breast tumours, originally reported according to 2007 guidelines, were re-scored using 2013 guidelines and the diagnostic categories compared. The results of ISH testing on a separate cohort of 32 breast tumours reported as Her2 IHC 2+ following the introduction of the 2013 guidelines, that would have been designated 1+ according to 2007, were reviewed.
Results: Application of 2013 guidelines resulted in a decrease in tumours classified as HER2 negative (83/251 vs 144/251) and a comparable increase in those classified as equivocal (2+) (139/251 vs 80/251). Relatively few tumours were re-classified as positive (29/251 vs 27/251). 3/ 32 breast cancer cases (HER2 IHC 2+ as per 2013 guidelines, 1+ using 2007 guidelines) were HER2 ISH positive.
Conclusion: Application of the 2013 guidelines increases the HER2 IHC equivocal (2+) category and requirement for reflex ISH testing. The reduced threshold for ISH testing identifies some patients with HER2 positive breast cancer whose tumours would have been categorised as HER2 negative according to the 2007 guidelines.

Pathologic Response to Neoadjuvant Treatment and Biomarker Profile in a Breast Cancer Cohort

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Purpose of Study: Extent of tumour response to neoadjuvant therapy for breast cancer is variable and correlates with patient and tumour characteristics.
The aim of this study was to evaluate pathologic response to neoadjuvant treatment and correlate response to tumour biomarker profile in a breast cancer cohort.
Methods: Sequential neoadjuvant treated breast cancer cases were identified over the study period. Biomarker profile was evaluated by IHC (ER, PgR, HER2) and Brightfield HER2 DNA in situ hybridisation (Ventana Inform).
Pathologic response to treatment was evaluated and residual cancer burden (RCB) score and class (complete pathologic response (pCR), RCB1, RCB2 and RCB3) generated using an online tool (MD Anderson Cancer Centre).
Summary of Results: 123 patients with available biopsy and excision data were identified. Biomarker profiles (pre-treatment core biopsy testing) were as follows: Hormone positive, HER2 negative: 52.8%; Hormone positive, HER2 positive: 19.5%; Hormone negative, HER2 positive: 14.6%; Triple negative: 13%. Cases were categorised as pCR (14.6%), RCB1 (6.9%), RCB2 (36.9%) and RCB3 (41.5%). Results for combined pCR and RCB1 groups were: Hormone positive, HER2 negative: 10.8%; Hormone positive, HER2 positive: 33.3%; Hormone negative, HER2 positive: 44.4%; Triple negative: 18.8%.
Conclusions: Hormone positive, HER2 negative tumours were the largest group to receive neoadjuvant therapy in this study, however showed a low frequency of substantial pathologic treatment response. HER2 positivity correlated with highest frequency of complete or near complete pathologic response.

Clinico-Pathologic Characteristics in Neoadjuvant Breast Cancers: A Single Institution's Experience

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Aim: Increasing numbers of breast cancer patients are being treated with neoadjuvant therapy. Pathologic evaluation of post neoadjuvant surgical specimens offers a unique opportunity to assess tumour response to systemic agents and allows evaluation of post-treatment prognostic parameters. The aim of this study was to evaluate the clinico-pathologic features in a large cohort of neoadjuvant treated breast cancers.
Methods: All patients receiving neoadjuvant therapy followed by primary breast excision were identified from institutional files over a 62 month period. All specimens were processed in a single laboratory with uniform standardized pre-analytics, macroscopic and microscopic evaluation and calculations performed as per Residual Cancer Burden (RCB, MD Anderson Cancer Centre Calculator).
Results: 130 patients were identified who had received neoadjuvant therapy followed by breast excision over a 62 month period. Mean age: 51.7 years. 40% had wide local excision, 60% had mastectomy. Core needle biopsy histologic subtype; ducal carcinoma 82.5%, lobular carcinoma 9.7%, metaplastic carcinoma 2.9%, mixed ductal and lobular carcinoma 2.9%, mucinous carcinoma 1.9%. Core needle biopsy tumour grade; grade 3 60.8%. Tumour grade changed between biopsy and excision in 27%, downgraded in the majority (77.8%). Pre neoadjuvant hormone and HER2 status; 52.9% hormone positive/HER2 negative, 19.5% hormone positive/HER2 positive, 14.6% hormone negative/HER2 positive, 13% triple negative. Hormone and/or HER2 status changed in 33.7% post-neoadjuvant cases. Of these, PR changed in 25.3%, ER changed in 9.6% & HER2 changed in 7.2%. The RCB classes were calculated as complete pathologic response (pCR), RCB1, RCB2 & RCB3 in 14.6%, 6.9%, 36.9% & 41.5%, respectively.
Conclusions: In our institution the majority of patients chosen for neoadjuvant therapy are Grade 3, ER positive/HER2 negative tumours. Tumour grade changed in 27%, hormones and/or HER2 changed in 33.7% and 14.6% had pCR.

Invasive Breast Cancer in Women over 75: An Evaluation of Predictive Markers and Aggressive Subtypes

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Purpose of the Study: Breast cancer is a common disease of the elderly with incidence increasing with age. Many studies site increased comorbidities, higher stage at diagnosis and less effective treatment as the reasons for poorer survival rates.
The aim of our study was to evaluate poor prognostic parameters including histologic grade and predictive markers in elderly women with invasive breast cancer.
Methods: All patients aged 75 years and over at the time of diagnostic breast core needle biopsy were identified from the institutional files of a tertiary referral subspecialised breast service over a 5year period (2010-2014). All core needle biopsies were taken from the primary breast cancer and processed in a single laboratory with standardised uniform pre-analytics. Hormone and HER2 evaluation with IHC and Brightfield dual in situ studies were performed as per UK recommendations.
Results: 1603 patients were diagnosed with breast cancer during this time period. 374/1603 (23.3%) were 75 years or over at the time of diagnosis. 348/374 (93%) had invasive carcinoma. Age range for these 348 patients was 75-99 years with a mean age of 81.9 years. Histologic subtypes; 76.2% ductal carcinoma, 18.7% lobular carcinoma, 2.3% mutinous carcinoma, 1.1% papillary carcinoma, 0.9% mixed ductal and lobular carcinoma, 0.4% micro papillary carcinoma and 0.4% mixed tubular and cribriform carcinoma. Histologic grades; 30.7% grade 3, 58% grade 2, 11.3 % grade 1. HER2 profile; 86.3% HER2 negative, 13.6% HER2 positive. Triple negative tumours; 9.2%.
In total, 36.8% tumours were either grade 3, HER2 positive or triple negative.
Conclusion: Our study showed that over one third (36.8%) of women with breast cancer diagnosed over the age of 75 had poor prognostic parameters including grade 3, HER2 positive or triple negative tumors.

Mitotic Activity in Benign and Malignant Fibroepithelial Lesions

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Introduction: Classification of fibroepithelial lesions into fibroadenoma, cellular fibroadenoma and phyllodes tumour is challenging and based on features including stromal cellularity, stromal mitotic activity, stromal overgrowth and cytological atypia. Brisk mitotic activity is associated with a diagnosis of phyllodes tumour. The proportion of benign otherwise typical fibroadenomas bearing low level mitotic activity is not well documented.
Aim: To document mitotic activity in a series of fibroepithelial lesions.
Methods: The laboratory information system database was searched for cases coded as 'Fibroadenoma' and 'Phyllodes tumour' from 2008 to 2013. Retrieved case reports were analysed for diagnosis, mitotic activity, tumour border, stromal cellularity, stromal overgrowth and the presence of malignant heterologous features. The data was compiled and analysed with Microsoft Excel.
Results: 1233 fibroepithelial lesions were retrieved and analysed and 64 mitotically active lesions were identified (5.2%). Of these, 50% were fibroadenoma (FA), 22% Benign Phyllodes Tumour (PT), 16% Borderline PT & 12% Malignant PT. Mitotic activity was higher in PT than FA (56% vs. 0.07 % > 4/10HPF) but FA did have mitotic activity (0.39% >2/10HPF). Average age at diagnosis was higher for malignant PT than FA (49 vs 39 yrs). Tumour borders were well circumscribed for FA and benign PT (66 and 86%) in contrast to infiltrative foci in borderline and malignant PT (75 and 71%). While stromal cellularity was noted to be increased in most lesions, stromal overgrowth was more likely to occur in malignant and borderline PT (100%) than in FA and benign PT(38 and 55%).
Conclusion: Occasional mitotic figures may be identified in benign fibroepithelial tumours, including fibroadenomas. Mitotic activity should be considered with other parameters to minimise the risk of misclassification.

An Audit of Breast Cancer ER Positivity Reporting Rate in Wales

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Background: Immunohistochemical oestrogen receptor status (ER) is an important index of tumour prognosis and treatment selection for breast cancer patients. Inaccurate ER analysis has serious clinical implications particularly since a false-negative result leads to patient not receiving the highly effective anti-oestrogen treatment. Following concerns reported in 2012 regarding under reporting of ER status at a UK centre, an audit of Wales laboratories routinely testing ER was conducted. Aims and Objectives: To determine ER positivity rates in Welsh National Health Service (NHS) laboratories to identify any under reporting.
Methods: ER results reported at 5 Welsh NHS laboratories (A-E) in 2012 were retrospectively assessed and sub-divided into screen detected (SD), symptomatic (S) or recurrent/metastatic (RM).
Results: The numbers and respective ER positivity rates (%) of SD, S & RM cases for each centre were determined to as follows: A: 210 (97.1); 235 (89.4); 0 (N/A); B: 94 (89.3); 212 (77.8); 2 (50); C: 178 (93.8); 245 (88.5); 0 (N/A); D: 64 (96.9); 494 (92.9); 16 (50); E: 0 (N/A); 91 (81.6); 7 (57.1). The average ER positivity rate of 89.8% for primary tumours is comparable but higher than UK-wide average rate of 83.7% (UKNEQAS audit). The reason for this higher rate is not certain but may relate to methodological or demographic differences.
Conclusions: 1) There is no evidence of under reporting of ER in Wales; 2) screen detected cancers have a higher ER positivity rate compared to symptomatic and recurrent/metastatic cancers as expected.

Audit of Fibroepithelial Tumours in a Nigerian Tertiary Institution

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Fibroepithelial lesions are the commonest lesions of the breast seen in our laboratory consisting of fibroadenomas and Phyllodes tumours (PT). PT is a rare fibroepithelial breast neoplasm accounting for 0.3-0.9% of all primary breast tumours. The aim of the study was to audit all fibroepithelial lesions and to reclassify all confirmed cases of Phyllodes tumour seen in the study period according to the criteria proposed by World Health Organisation (2003). Records and slides of fibroepithelial lesions of the breast received at the department between January 2008 and December 2013 were retrieved and reviewed by the authors. Out of the 1242 fibroepithelial lesions of the breast retrieved, all but 19 were fibroadenomas. The 19 were initially reported as PT: 11 benign, 2 borderline, 2 malignant and 4 unclassified; however only 16 of these 19 PTs (84%) met the WHO criteria on review. The remaining 3 (16%) turned out to be fibroadenomas based on the absence of stroma overgrowth and hypercellularity. The PTs were reclassified into benign PT, borderline PT, and malignant PT accounting for 75% (12/16), 18.7% (3/16) and 6.3% (1/16) respectively. All of the PTs previously unclassified turned out to be benign PTs. One of the borderline PTs was originally reported as malignant PT. All cases initially diagnosed as fibroadenomas did not change on review. These results show that fibroadenomas are rarely misdiagnosed. The 3 cases misdiagnosed as phyllodes may have been prevented if standard data sets were in use. Cases simply referred to as PT without further classification, limit the patients' access to appropriate management as accurate classification helps in the overall management and prognostication.

A Review of Columnar Cell Lesions in Fibrocystic Changes of the Breast in a Tertiary Centre; A 10-Year Retrospective Study

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Columnar cell lesions (CCLs) of the breast are now gaining clinical attention because of the increasing use of mammographic screening as one of the breast cancer prevention strategies. CCLs are important because certain variants may be precursors of low-grade ductal carcinoma in situ (DCIS). In Nigeria and much of the developing world, mammography is novel and CCLs have not been studied among the black populations as a result. The aim of this study was to estimate the occurrence of CCLs in Nigerian women using fibrocystic changes as a surrogate lesion as well as determining the range of CCLs found using standard histologic criteria. The slides and records of all consecutive breast biopsies that were diagnosed with fibrocystic changes (FCC) within a 10-year period at the department were reviewed by the authors. Thirty cases of CCLs were identified among the 559 cases of FCC found. The ages of the patients ranged from 16years to 63years, the age group with the highest incidence was in the 5th decade accounting for 26.7% while the least incidence is in the 2nd decade of life accounting for 9.8%. The commonest pattern found was Columnar Cell Change (CCC) at 66.7%, Columnar Cell Hyperplasia at 16.7%, CCC and CCH occurring together at 16.7%. No case of flat epithelial atypia (FEA) variant was found. This study shows that CCLs occur among Nigerian women and by extension the Black women population. The FEA variant, the putative DCIS precursor, was not found among our cohort, probably because of the restriction of study material to fibrocystic changes. The age distribution of our patients was similar to those reported among Caucasian women. We recommend that larger studies be carried out when mammography becomes more widely available.

Molecular Subtypes and Prognosis in Two Cohorts of Norwegian Breast Cancer Patients

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Purpose: Our group previously subtyped tumours from a historic cohort of Norwegian breast cancer patients born 1886-1928, with limited access to adjuvant treatment and mammography screening. The purpose of this study was to compare the distribution and prognosis of these subtypes with a second cohort of breast cancer patients born 1917-1972 from the same geographical area.
Methods: Tissue microarrays were constructed from archival formalin-fixed, paraffin-embedded tissue from 909 (Cohort 1) and 514 (Cohort 2) cases. Using IHC and ISH as surrogates for gene expression analysis, tumours were classified into six molecular subtypes: Luminal A, Luminal B (Her2 negative), Luminal B (Her2 positive), Her2 type, 5 Negative Phenotype (5NP) and Basal-like phenotype (BP). Proportional hazards models were used to assess prognosis.
Results: Mean age of diagnosis was higher in cohort 1 (72.5 vs 58.3 years). 47.6% (Cohort 1) and 52.9% (Cohort 2) of tumours were Luminal A. Survival increased for all subtypes in cohort 2. For the first five years after diagnosis, risk of death from breast cancer was highest for Her2 type and 5NP in cohort 1 (age-adjusted hazard ratio 4.4 and 3.2 when compared with Luminal A), and for BP and Her2 type in cohort 2 (age-adjusted hazard ratio 10.5 and 7.5). Mean age for BP cases was lower in cohort 2 (53.2 vs 71.7 years). After the first five years no significant differences in risk of death were found between subtypes.
Conclusion: Cohort 2 comprised younger women with a higher proportion of Luminal A tumours, and with improved survival for all subtypes. BP appears to imply a relatively poorer prognosis among younger women compared to older women. Molecular subtyping gives prognostic information the first five years after diagnosis.

A Pathological Review of Parathyroid Carcinoma

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Background: Parathyroid carcinomas are rare tumours with incidence of 0.5-4% of all the cases of primary hyperthyroidism. The Royal College of Pathologists (RCPath) minimum dataset (2010) has identified a number of features which help in establishing a diagnosis of malignancy. The current study aims to review all parathyroid carcinomas diagnosed at Nottingham University Hospital and Sherwood Forest Hospital, Sutton-in-Ashfiled, between 2004 and 2014.
Material and Methods: Pathology computer databases were searched at both hospitals to identify all parathyroid carcinomas diagnosed during study period. Reports were assessed against RCPath minimum dataset items suggestive of malignancy.
Results: Overall 9 patients were diagnosed as parathyroid carcinoma (7 females, 2 males). The age ranged from 29 to 70 years (median 56years). Macroscopically, specimen weights ranged from 0.7g to 27.5g. The tumour size varied between 15mm and 46mm (median35mm). Documentation of various features of malignancy was as follows; thick fibrous capsule 3/3, dense fibrous septae 7/7, diffuse sheets 3/3, nuclear monotony 3/4, frequent mitoses (>1 per 10hpf) 2/2, vascular invasion 7/9, peri-neural invasion 0/9, direct extension into adjacent soft tissues 7/8, capsular invasion 7/7. Predominant growth patterns were; diffuse sheets (4), irregular islands (2), trabeculae (1), lobules (1) and solid (1). Ki-67 labelling index (reported in 4 cases) ranged from 1% to 3.9%. None of the cases showed abnormal mitoses.
Conclusion: This review shows that there is room for improvement in documentation of features of malignancy in parathyroid carcinoma. We are reviewing the original slides to correlate histological features with current RCPath dataset items.

Snomed Code M09350; 'Morphological Description Only' – A Six Month Review

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Purpose of the study: The SNOMED coding system is used to designate T (Topography), M (Morphology) and P (Procedure) codes. This system is invaluable in the audit of workload and as such accurate coding is imperative. The aim of this review was to examine the usage of the morphological code M09350, designated as a "morphological description only", over a six-month period within a single histopathology department. The objective was to assess the appropriateness of cases for which this M Code was designated.
Methods: A computerized search was performed and the authorized cases coded with the M code 09350 were reviewed. Descriptive reports, without definitive diagnosis were deemed appropriately coded. Cases were deemed inappropriate if an alternative specific M code was available.
Summary of results: In total 160 cases were coded with M09350 in the six month period. 53% of cases were deemed to be inappropriate. 10% of inappropriately coded reports described reactive or regenerative changes. 11% of the coded specimens were limbs referred to the department for safe disposal. 7% of cases described squamoproliferative lesions. With 1% comprising thyroid cytology specimens and 2% bone marrows showing a myeloproliferative disorder. The more commonly coded specimens included skin (28%) and bone marrow (13%), reflecting the fact that reports from these sites commonly require descriptive commentaries with a definitive diagnosis depending on clinicopathological correlation.
Conclusions: The morphological code m09350 should be used for specimens where a morphologically descriptive report is issued and no specific diagnosis is made.
Coding can be improved by education of pathologists and introduction of more specific codes in problem areas such as thyroid cytology, disposal cases, myelodysplasia, squamoproliferative lesions and regenerative changes.

When Are We Picking up the Phone? An Audit of Critical Value Reporting

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Purpose of the Study: In 2013 The Royal College of Pathologists issued guidelines on the communication of urgent reports or unexpected findings. Numerous studies have looked at the concept of critical value reporting in pathology however it has proven to be a difficult model to apply to the myriad of pathological diagnoses. This audit aimed to assess the cases which were communicated directly to the clinical teams.
Methods: Computerised laboratory database was retrospectively reviewed for reports coded as having been phoned directly to clinicians. All such reports were examined to identify clinical features, diagnosis and comments which may indicate a critical or unexpected diagnosis.
Summary of Results: 607 telephoned reports were identified in the interval between January 2011 and August 2014, out of a total of 114320 cases. Malignant cases accounted for 57.3%, frozen sections for 9.7%, and cytology for 7.7%. While the majority of calls related to the routine communication of results and requests for clinical information, 25 unexpected or critical events were identified. These included 5 cases of delayed malignant diagnosis, 5 cases of frozen/permanent disagreement, 4 products of conception without villi, 3 cases of vasculitis, 2 cases of suspected perforation with fat in a biopsy sample, and 2 cancers upstaged following additional levels.
Conclusions: This audit showed that 0.5% of cases were telephoned directly to clinicians, this may be an under representation as the coding system is user dependent. Establishing local guidelines on critical cases may facilitate more efficient communication of urgent and unexpected results to our clinical colleagues.

Mediastinal Biopsies: Small Biopsies for Challenging Cases – A Single Centre Review

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Mediastinal masses comprises a varied and mainly neoplastic lesions arising from intra-thoracic organs. In 2011 guidelines for the interpretation of mediastinal biopsies have been published but it still remains challenging. We present data from a review of the mediastinal biopsies reported in the last two years within our department. We have reviewed 95 consecutive mediastinal biopsies from January 2012 to December 2014. We compared each case preliminary and final diagnosis after performing special stains, molecular analysis or referring the case. Also, when additional material was available, we compared the diagnosis of both samples to find any discrepancy, especially when the first specimen was sent for frozen section examination. For those cases with no available follow up, we reviewed the slides to assess the accuracy of the diagnosis. 46 out of 95 cases had further material for comparison and there was no discrepancy between the two diagnoses. 34 frozen section reports were all confirmed on paraffin sections. 49 cases had no further material available for diagnosis, however 17 were sent for expert opinion and our diagnosis was confirmed. The remaining 32 cases were reviewed by two pathologists and there was independent agreement in all cases. 12 cases out of 95 showed no discrepancy in the diagnosis after review, however they were considered overall not diagnostic after comparison with further material: 2 cases were frozen sections with resection specimen immediately available, 10 cases were small biopsies and additional tissue was sent within few weeks. 6 cases were not diagnostic because insufficient amount of tissue (3 to 15mm) resulting in difficult processing whilst sampling error occurred in the other 6 cases. Mediastinal masses often pose a diagnostic challenge for pathologists. Our data show that a correct diagnosis is often reached on biopsy (87.4% viewed as adequate). However good sampling is essential as non-diagnostic/inadequate levels were 12.6%.

Investigation of a Rapid Reliable Method for Tumour Percentage Estimation for Diagnostic Molecular Analyses

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Background: Tumour percentage estimate (TPE) is important index for deciding adequate tumour presence in tissue sections submitted for diagnostic molecular analysis. The commonly employed crude visual (microscopic) estimate method is rapid but subject to inter-observer variation and may be grossly inaccurate for tissues with sparse tumour population. Stereological counting methods are reliable but can be labour intensive and time consuming.
Aim: Investigation of reliability and time efficiency of stereological grid point counting method for routine tumour percentage estimation.
Methods: A point lattice (25 dots x 52 dots 0.8cm apart) outlined on a transparent sheet was superimposed on a desktop screen projecting the respective Aperio digital images (x20 mag) of H & E stained sections of 5 (A-E) different colorectal cancers. TPEs based on counts of tumour and stromal nuclei touching the point lattice at every 5th (5TPE) or 10th (10TPE) line were derived for a fixed area (41x19.8 cm) and compared with absolute TPE (ATPE) and crude TPE (CTPE).
Results: The CTPE and ATPE, 5TPE5 and 10TPE for the 5 cases (A-E) were calculated to be as follows, respectively: A — 40, 49.3 53.3, 54.1; B — 65, 62.5, 57.3, 57.1;; C — 60, 71.7, 73.7, 73.8; D — 70, 72.6,74.8, 73; E — 90, 89.9, 91.2, 90.6. The average time taken to obtain these 2, 30, 7.5, 15 min, respectively.
Conclusion: The lattice grid point counting is potentially more reliable the crude visual for tumour percentage estimation, meriting investigation of a grid point lattice directly superimposed on the microscopic image as a more direct alternative stereologically sound cell counting method.

Ensuring Quality in Histopathological Reporting of Laparoscopic and Open Colorectal Cancer Resections

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Purpose: With increased use of laparoscopic procedures over open surgery in colorectal cancer resection we need to ensure that pathological reporting standards can still be met with specimens from laparoscopic surgery, even with smaller mesenteric samples. We audited the histopathology reporting of laparoscopic and open colorectal cancer resections against the minimum standards in The Royal College of Pathologists (RCPath) published dataset guidelines.
Methods: Patients who underwent colorectal resection for malignancy over a twelve month period were identified through clinical coding. The list was verified using the pathology report and incorrectly coded patients excluded. Laparoscopic procedures were identified and an equal number of open resections were randomly selected. Pathology reports were analysed as to; type of resection, number of lymph nodes examined, serosal involvement, tumour stage, extramural venous involvement. Differences between the two groups were analysed using a Student's t-test. A significance level of $p<0.05$ was used throughout.
Results: Thirty seven lapraroscopic resections and 35 open resections were analysed. A greater proportion of right hemicolectomies were laparascopic. Mean number of lymph nodes sampled for laparoscopic and open procedures were 16.3 and 17.1 respectively. Serosal involvement was 17.6% and 16.1%. Incidence of extramural venous invasion was 40% for both. No significant difference was noted between the groups.
Conclusions: Although the sample sizes are small, there was no difference between laparoscopic and open surgery in meeting the RCPath standards. This supports the increased use of laparoscopic surgery in colorectal cancer. There is a lower incidence of serosal involvement in these resections than that recommended in the dataset. This may be due to earlier detection of tumours following the introduction of the Bowel Cancer Screening Programme.

An Audit of Malignant Melanoma Histopathology Reporting After Proforma Introduction

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Purpose of the study: This audit's objective was to determine whether malignant melanoma reporting was including the core criteria as expressed in the minimum dataset requirements of the *Royal College of Pathology Dataset for the Histological Reporting of primary cutaneous malignant melanoma (3rd Edition)* and if this had improved since the introduction of a compulsory proforma.
Methods: All histological diagnoses of malignant melanoma at the hospital over two 12 month periods were identified using SNOMED codes on the pathology database pre and post the compulsory proforma.
Summary of results: Within the hospital a reporting proforma is available with the microscopic core-criteria. During the year a national electronic proforma has been introduced including both macroscopic and microscopic core criteria. 93% of reports used a proforma compared with 66% in the previous study with an overall improvement of core-criteria inclusion from 69% to 93% as well as 70% of reports now including TNM staging compared with 0% previously. Overall there was a discrepancy between the inclusion of macroscopic (87%) and microscopic (97%) core criteria.
Conclusions: There was a significant improvement in the overall core-criteria inclusion percentage since making proforma use compulsory. There is still room for improvement when reporting macroscopic criteria, the use of the national proforma, (which includes this and had started to be used towards the end of this study) aims to improve this.

Educating in Healthcare – How we can do it Better

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There is evidence that the quality of education and the environment in which it occurs in the NHS are in need of improvement (Francis, Berwick Reports). The UK General Medical Council reports tensions between "service" and "education/teaching/training". An educational environment is the relationship of learning with the people who are learners in their physical temporal and social surroundings. Do we attend to the educational environment to provide learners and teachers with the best outcomes for sustainable laboratory services? We suggest not. This paper is a synthesis of data from multi-disciplinary workshops in conferences (London, NACT; Milan, AMEE) in 2014, involving 59 and 34 participants respectively. These were doctor-educators and academics with pedagogical interest. It also draws on a discrete choice experiment (DCE) with 1323 trainee participants surveyed across specialties in Scotland and the north of England in autumn 2013. Workshop data demonstrate several themes. Problems include a dichotomy between the accepted "continuum of education" and processes involved in its parts. Employers' expectations do not match those of trainees. It is hard to translate educational ideas into practice, relying on individuals who succeed despite rather than because of institutional support. Medicine relies on communication and human interaction: educational relationships are challenged and role models fail to invigorate and enthuse. Solutions encompass the need to generate a culture in healthcare that values, normalises and prioritises education. Fostering educational relationships and conscious socialisation may facilitate this as may transparency in funding. The DCE highlights the importance trainees place on good working conditions while making career choices, above good programmes and nice places. In considering future diagnostic services, it may be worthwhile to note the evidence and have the people placed in an educational culture at the heart of plans.

Investigation of the Role of Alcohol in Intestinal Cancer

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Background: Alcoholic beverages have been classified as carcinogenic, with tumour sites related to alcohol consumption including the colorectum and upper aerodigestive tract (IARC 2007). A variety of mechanisms may be causal for the enhanced cancer risk linked to alcohol consumption, including direct genotoxic effects of ethanol and its metabolite acetaldehyde (AA). The relevance of these mechanisms in the colorectum has not been fully elucidated yet, neither has the role of different isoforms of alcohol metabolising enzymes. While Aldh2, the main enzyme for AA detoxification in the liver, has been investigated widely, little is known about Aldh1b1 which also has a high affinity for AA and is highly expressed in the GIT.
Aims: We aim to further elucidate the mechanisms of alcohol as a risk factor for cancer in the intestines, focussing on alcohol metabolising enzymes and DNA repair, and on genotoxic effects.
Methods: 20% (v/v) ethanol was administered to mice via drinking water for 3 weeks, while control groups received normal drinking water. Swiss rolls of the intestine were prepared for immunohistochemical analysis and intestinal epithelium was isolated.
Results: While Aldh2 was uniformly expressed in the intestinal epithelium, Aldh1b1 expression was preferentially located in the crypt bases. Cyp2e1 was up-regulated by alcohol in the liver, but not expressed in the intestines. Alcohol increased the number of Ki67-positive cells in the liver and small intestine. Enhanced phospho-γH2AX and p53 were observed in the colon, indicating DNA damage.
Conclusions: We have established a protocol for short term treatment of mice with ethanol and we demonstrated DNA damage and enhanced proliferation after 3 weeks of ethanol treatment. This provides a good basis for further elucidation of the genotoxic mechanisms of alcohol and its metabolites, as well as the protective mechanisms involved.

Cytokine Networks in Endometrial Carcinogenesis

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Endometrial cancer (EC) is the commonest gynaecological malignancy in the developed world and falls into two categories. Type I (~75% cases) is generally oestrogen-sensitive, develops from premalignant hyperplasia and is low-medium grade. By contrast, the rarer high grade Type II is frequently oestrogen receptor negative, have no premalignant lesion and have poorer outcomes. This study characterised cytokine-based microenvironmental features associated with endometrial carcinogenesis given their role in immunoregulation and tumour behaviour. Endometrial lysates (38 normal, 25 hyperplastic and 97 cancerous; 46 Type I, 51 Type II) were profiled for 49 cytokines by multiplex immunoassay which were then standardised against total protein. Data were analysed by Kruskall-Wallis tests with Mann-Whitney-*U* tests *post hoc*, applying False Discovery Rate correction for multiple comparisons. The *R* package *Catnet* was used for learning categorical Bayesian networks, where data were fitted according to Maximum Likelihood Estimation-based network search by Simulated Annealing without a prior seed network. Final networks were selected based on maximal Akaike information criterion values and visualised in Gephi. Significant differences in the concentration of 28 cytokines were noted between the groups. Cytokine profiles matched the robust histology-based discrimination between normal, hyperplastic and cancerous endometrial tissues, including differentiating between Type I/II cancers. These differences were also reflected in cytokine interrelationships; distinct subnetworks with different nodal foci were seen across all groups. In particular, GM-CSF appeared to play a major regulatory role uniquely in normal endometrium. However, the hub node function of other mediators was more conserved: IL-17 was consistently found in this capacity in all categories except Type II ECs, which instead favoured IL-4 and IFN-γ. Moreover, a central role for agents such as TNF-β was only seen in cancers.

Histopathologic Assessment of Testicular Specimen in a Nigeria Tertiary Hospital

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Introduction: Removal of testicular tissue is often performed for the evaluation or treatment of testicular lesions. Histopathologic assessment of such specimen is therefore useful in the review of current urological practice. Testicular biopsies are also of vital importance in the management of infertility.
Aim and objectives: This study aims to characterize the histologic spectrum of testicular lesions as well as relating them to the various surgical procedures by which they were taken.
Methodology: Records of all testicular specimen received in the department from 2005 to 2014 were retrieved. Data such as age, indication for biopsy, nature of surgical specimen and histologic diagnoses were extracted. They were classified, analyzed and represented in tables and charts.
Results: A total of 167 testicular and paratesticular specimens were submitted during the study period constituting 0.7% of surgical specimen received during the study period. The most common indication for the submission of testicular specimen was for the treatment of prostate cancer (43.7%) followed by pain (20.8%) and the presence of a mass (19.6%). Orchiectomy specimens were the commonest samples received (76.8%). A significant proportion of cases 56.25% had orchiectomy for benign lesions. Hypospermatogenesis with maturation arrest (57.8%), hypospermatogenesis (15.8%) and tubular hyalinization (15.8%) were the common histologic diagnosis of male infertility. Germ cell tumours were the commonest testicular neoplasms (62.5%). Embryonal rhabdomyosarcoma was the only paratesticular malignancy seen.
Conclusion: Treatment of prostate of cancer was the commonest indication for testicular biopsies in our environment. Testicular tumours are not common. Fifty six percent of the cases had orchiectomy for benign lesions which call for a review of practice. With core needle biopsies and frozen section analysis, unnecessary orchiectomies can be avoided.

An Interesting Case of Persistent Mullerian Duct Syndrome- Rare Female Variant

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Persistent Mullerian duct syndrome is a rare form of internal male pseudohermophroditism characterised by the presence of Mullerian duct derivatives such as cervix, uterus and tubes in a phenotypically and karyotypically male patient (46,XY). Only 150 cases have been reported in the literature so far with two types of variants described. The more common male form accounts for 80-90% of the cases and is characterized by unilateral cryptorchidism and contralateral inguinal hernia containing uterus and fallopian tubes. The female form accounts for only 10-20% the cases where in there is bilateral cryptorchidism with the testis fixed within the round ligaments in an "ovarian" position inside the pelvis.
We report a 49-year-old gentleman diagnosed with the rare female variant of persistent Mullerian duct syndrome. He presented with bilateral cryptorchidism in childhood and was further lost to follow up. He re-presented with symptoms of left ureteric calculus and underwent a computed tomography. This demonstrated a saccular structure anterior to the bladder with bilateral tubes leading onto a pair of intraabdominal gonads. Further investigations revealed a 46,XY karyotype in the gentleman. A surgical excision of the remnant structures was then performed with histopathology confirming the presence of primitive cervix and uterus along with a pair of fallopian tubes representing Mullerian duct remnants. The gonads present in the "ovarian" position were confirmed to be testes exhibiting features of testicular dysgenesis and changes secondary to cryptorchidism. A pair of spermatic cord ran alongside the fallopian tubes in the same sheath and a pair of seminal vesicles on either side of the primitive cervix was also found. To have such well-formed male and female genital organs running alongside each other interconnected as in this case is believed to be rare.

Re-audit of Prostate Needle Core Biopsy (NCB) Reports with Adenocarcinoma

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Purpose of study: Re-audit of an original project performed in 2011 which assessed reporting of prostate NCB with adenocarcinoma. The results from 2011 showed that although there was good compliance for reporting important prognostic criteria (dictated by the RCPath dataset), there was variability between different pathologists within the institution. The main recommendation was to use an agreed standard template for all NCB specimens with cancer to ensure consistency in reporting so that high standards were maintained. This re-audit will evaluate whether the recommendations have been met and whether this has improved reporting standards.
Methods: A retrospective computer search identified all prostate NCB containing adenocarcinoma from October 2011 to October 2013. Fifteen reports by each consultant were then randomly chosen from the list for assessment (105 total). Each report was examined against the following criteria:
• Specimens submitted according to local protocol
• Use of standard template
• Grouping of cores according to side of prostate
• Localisation of cores involved with cancer: apex, mid or base
• Gleason Score given
• Correct terminology used
• Reporting tumour extent: Number of cores involved by adenocarcinoma as a fraction of total number submitted from that side; total percentage cancer involving each side
Results: 100 % compliance was achieved in 6/8 variables. Improvement in compliance with all criteria was also seen.
The two variables where the results were short of 100% were:
• Use of standard template; (99% achieved): An individualised report was required in one case where material did not survive processing.
• Specimen submitted according to local protocol; (96% achieved - 4 cases)
Conclusion: The original audit recommendation of using a standard template for prostate NCB has been implemented well and and has provided significant improvement in reporting standards. Important prognostic criteria are now included with less variability and greater consistency.

● ***Within the hospital 'in house' publication for the 9th Mid Cheshire Hospital Foundation Trust (MCHFT) Healthcare Exposition "Evidencing Quality"***

Audit of the Reporting of Testicular Neoplasms within a University Teaching Hospital

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An updated dataset guiding the reporting of testicular neoplasms was issued by the Royal College of Pathologists in May 2014. This defined core items that need to be represented in all histological reports of testicular neoplasms. Several of these core dataset items were adjusted from previous dataset published in October 2007. Examples of these adjusted items included rete stroma and hilar soft tissue invasion and documenting which tumour type showed lymphovascular invasion in mixed germ cell tumours. All testicular neoplasm reports from a tertiary centre within an 18 month period from July 2013 to December 2014 were analysed. A total of 67 cases were identified of which were 61 germ cell tumours and 6 were non germ cell tumours. Compliance of the core items were analysed by comparing report contents with the Royal College of Pathologists guidelines operational at the time of reporting. There was good compliance with items such as type of tumour 61 (100%), lymphovascular invasion 61(100%), intratubular germ cell neoplasia 61(100%). rete stromal invasion was mentioned in 29/31(94%) of cases reported after introduction of the new dataset. There were areas for improvement, including the reporting of core data items that have remained unchanged from previous guidance, such as maximum tumour diameter 57/61(93%) and spermatic cord invasion 48/61(78%). Identification of such areas for improvement allows action to be taken to ensure that pathology reports meet the minimum standards recommended by the Royal College of Pathologists.

Audit of Seminal Vesicle Invasion in Radical Prostatectomy Specimens, Do We Really Need to Process the Lot?

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Purpose of the Study: Seminal vesicle invasion (SVI) in radical prostatectomy specimens has a poor prognosis and is staged pT3b in the UICC 7th edition. No universally accepted guidelines currently exist on the embedding of seminal vesicle (SV) tissue. We aimed to audit our incidence of pT3b reporting and in these cases to look at SVI-type (Type 1-3) and SV sampling method, with a view to moving from complete to partial embedding.
Methods: 258 radical prostatectomy cases over a four year period were identified. These specimens were entirely embedded. Cases reported as pT3b were re-reviewed to confirm SVI and the SVI-type documented.
Results: 16 of 258 cases were reported as pT3b (6.2%). Of the 14 cases where it was possible to determine SVI-type, 13 cases (92.8%) showed type 2 invasion and in all of these cases invasion was confirmed in either the slice of the junction of the base of prostate and SV (junctional slice) or the most proximal SV section. In the one case (7.2%) of type 3 invasion the proximal and mid SV and the junctional slice were negative for tumour invasion. However, lymphovascular invasion (LVI) was identified in the prostate in this case and confirmed with immunohistochemistry. Block numbers for SV tissue averaged 5.6 regular blocks or 1.5 megablocks per case.
Conclusion: Our incidence of stage pT3b disease of 6.2% is within the expected published range (6% - 19.3%). Our results support a partial SV embedding technique sampling only the junctional slice or the most proximal slice of the SV, rather than mandatory blocking of all the SV tissue routinely, as tumour infiltration of the mid and distal SV is rare in the absence of involvement of the proximal SV. However, the entire SV should be retrospectively blocked if LVI is identified in the prostate. Partial embedding should reduce costs and could also improve turnaround times.

Case review: Birt-Hogg-Dubé Syndrome

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Birt-Hogg-Dubé (BHD) syndrome is a rare autosomal dominant genetic disorder arising from germline mutations in the folliculin gene, mapped to the short arm of chromosome 17. It is associated with cutaneous lesions including fibrofolliculomas, spontaneous pneumothorax and renal tumours. We present the case of newly diagnosed BHD syndrome in a 45 year old Irish female. Histological examination confirmed multiple chromophobe renal cell carcinomas (RCC) and a hybrid oncocytoma/chromophobe RCC. There was also background oncocytosis, comprising multiple, tiny parenchymal micronodules of clear cells with chromophobe RCC-like morphology and immunophenotype. Mutation of the folliculin gene was confirmed by direct sequencing. Pathologists should be aware of the possibility of BHD syndrome in patients presenting at a young age, with multiple renal tumours with either chromophobe RCC, oncocytoma or hybrid oncocytoma/chromophobe RCC features, particularly if there is also background oncocytosis. This case is illustrative of the fact that chromophobe RCC-like cells with clear rather than oncocytic cytoplasm form part of the oncocytosis spectrum and this finding should always prompt appropriate genetic analysis for BHD syndrome

Defining the Rectum and Sigmoid Colon: Should We Abolish the Term 'Rectosigmoid' to Improve Outcomes in Colorectal Cancer?

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The risk of circumferential resection margin (CRM) involvement is confined to tumours of the rectum with the risk of peritoneal involvement increasing the further a tumour is located above the peritoneal reflection. There is no internationally accepted definition of the upper limit of the rectum, and the term 'rectosigmoid' is frequently applied to tumours in this area leading to confusion around the risks and whether radiotherapy can be given. The photographs from 331 abdominoperineal excision specimens were available for quantitation using Aperio ImageScope. Both fresh and fixed specimen images were included where available. The position of the anal verge, top of the sphincters, anterior peritoneal reflection, mesorectal apex (defining the limit of the mesorectum) and high vascular tie were identified and the distances between each point measured. The work was supported by a PathSoc bursary. There was wide variation in the length of the mesorectum in both fresh (median 172 mm, IQR 146 to 199 mm) and fixed (166 mm, 140 to 196 mm) specimens. The length of the anal canal also showed variation (fresh 66 mm, 49 to 78 mm; fixed 66 mm, 53 to 75 mm). The height of the anterior peritoneal reflection was lower in females compared to males (fresh 125 vs. 132 mm, p=0.288; fixed 111 vs. 126 mm, p=0.034). There is marked variability in the anatomy of the rectum between individuals and genders. This potentially affects the risk of either CRM or peritoneal involvement and whether radiotherapy could be offered. A fixed definition of the upper limit of the rectum for all patients is not helpful. This should be determined for individual patients on the basis of the MRI findings. The term 'rectosigmoid' should be abolished and more accurate definitions based on the position of the mesorectal apex and commencement of the sigmoid mesentery should be used to define the boundaries of the rectum and sigmoid colon and determine subsequent risks to the patient.

Pre-Treatment and Post-Treatment Epidermal Growth Factor Receptor Pathway Mutations in a Prospective Phase II Trial (NWCOG EXCITE) of Cetuximab-Containing Chemoradiation in Locally Advanced Rectal Cancer

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Pre-operative chemoradiotherapy (CRT) with anti-EGFR antibodies may change the status of EGFR pathway mutations. We assessed the mutational status of a number of EGFR pathway genes before and after CRT in the NWCOG EXCITE trial. Patients with MRI-threatened surgical margins were given pelvic radiotherapy (45Gy) with capecitabine, irinotecan and cetuximab followed by surgery after 8 weeks. DNA was retrospectively extracted from the pre-treatment biopsy and resection specimen by macrodissecting areas of greatest residual tumour. The mutational status of KRAS (codons 12/13/61/146), NRAS (12/13/61), PIK3CA (542/545/546/1047) and BRAF (V600E hotspot) were determined by pyrosequencing. The work is presented on behalf of the NWCOG EXCITE trial investigators and was part-funded by a PathSoc fellowship. 80 patients commenced treatment and 76 underwent surgery with pathological complete response in 14 (18%) and near-complete in 6 (8%). Pre-treatment testing (n=78) detected mutations in KRAS (n=34), BRAF (n=3), NRAS (n=3) and PIK3CA (n=10). Any EGFR pathway mutation was detected in 58%. Following CRT, cases with residual tumour able to be tested (n=54) showed mutations in 32 patients (59%). There was a discrepancy compared to pre-treatment biopsy in 18 cases (33%): from wild-type (wt) to mutant (mut) in 9, from mut to different mut in 1 and from mut to wt in 7. One patient changed in 3 codons (mut to wt in KRAS 146/PIK3CA 545 and wt to mut in KRAS 12). In 12 patients (22%) this changed their overall EGFR pathway status (6x wt to mut and 6x mut to wt). Intratumour heterogeneity may explain some of the differences in EGFR pathway mutations reported between biopsies and resections presenting a challenge to personalised medicine. However, cetuximab may also drive the growth of undetectable mutant clones to detectable levels on pyrosequencing. Further assessment using more sensitive sequencing technologies is currently being employed to investigate these differences.

Investigating the Challenges of Using Historical Formalin-Fixed Paraffin-Embedded (FFPE) Material from the MRC CR07 Rectal Cancer Trial Using the Affymetrix OncoScan® FFPE Assay and Next Generation Sequencing

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There is a vast amount of historical FFPE material held in archives, but due to variations in fixation and processing this presents several challenges when applying newer genomic technologies to it. In this study we compared the genomic information obtained with the OncoScan® FFPE Assay Kit (OncoScan) and next generation sequencing (NGS).
Samples from 378 patients were obtained from 10 centres taking part in the MRC CR07 trial of short course radiotherapy versus selective long course chemoradiotherapy in rectal cancer. DNA was prepared using Agilent SureSelect kits and sequenced using Illumina platforms in parallel to analysis using the OncoScan assay. For both methods, quality control (QC) data was generated and the sample classified as a 'pass' if it fell within the pre-defined QC boundaries. For the OncoScan assay, copy number (CN) and somatic mutation (SM) data was further investigated. This study was part funded by a PathSoc Fellowship. In total, 272 cases (72%) passed the NGS QC and 232 (61%) passed the OncoScan QC. A total of 186 (49%) passed QC on both platforms with marked variability in sample pass rates between the 10 centres for the NGS (range 0% to 100%) and OncoScan (ranges 33% to 84%). When assessed manually, the OncoScan SM data was considered acceptable for 273 cases (72%), which included 40 initially classified as 'failed' by the QC data. Similarly, the OncoScan CNV data was interpretable for the majority of cases. This study has shown that whilst historical DNA held in the FFPE blocks of archival clinical trials like MRC CR07 can present challenges when using new genomic technologies, a large proportion of samples can still yield valuable genomic data. Marked variation exists in the quality of genomic material between centres confirming that differences in specimen handling affect DNA quality. Prospective trials must address this by standardising fixation and processing protocols.

The Effect of Multidisciplinary Education on the Quality of Colon Cancer Resection

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The plane of colon cancer resection has recently been shown to predict survival. Complete mesocolic excision (CME) with central vascular ligation (CVL) produces an oncologically superior specimen and appears to be related to optimal outcomes. We aimed to assess whether a regional educational programme in CME with CVL led to an improvement in the quality of colon cancer specimens. Following a regional educational programme in CME with CVL in the Capital and Zealand areas of Denmark, 686 cases of primary colon cancer resected across six hospitals were assessed by grading the plane of surgery and undertaking tissue morphometry. These were compared to 263 specimens resected prior to the educational programme. This work was partly supported by a PathSoc undergraduate bursary. Across the region, the mesocolic plane resection rate improved from 58% to 77% (p<0.0001). Hillerød hospital had implemented CME with CVL as standard prior to the educational programme and continued to produce optimal specimens. Three of the other hospitals showed a significant improvement in the plane of surgical resection. Hillerød specimens continued to be more radical with a greater distance between the tumour and the high tie, area of mesentery and lymph node yield compared to the other five hospitals. A multidisciplinary regional educational programme in CME with CVL has improved the oncological quality of colon cancer specimens as assessed by mesocolic planes, however, there has been no significant effect on the amount of tissue resected. Surgeons at Hillerød continue to produce more radical specimens suggesting that such educational programmes are not alone sufficient to increase the amount of tissue resected around the tumour. Hillerød have recently published their long term outcomes with survivals being 10% higher when compared to other hospitals across the region. Further engagement is now necessary to ensure that optimal outcomes are achieved across the region.

Investigating the Faecal Microbiome in Formalin Fixed Paraffin Embedded (FFPE) Material

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Purpose: Research into the faecal microbiome has shown a diverse population with a high level of variability between individuals. Altered faecal microbiomes are present in a range of diseases but work remains to understand their role in gastrointestinal disease. Current research into the microbiome makes use of fresh or frozen faecal samples. This restricts researchers to predominantly prospective study designs. One potential method for rapidly increasing and diversifying research is the retrospective study of FFPE material. We aimed to investigate the feasibility of typing the microbiome in FFPE faecal samples using next generation sequencing (NGS) technology.
Methods: Material from six faecal samples was divided and stored as frozen or fixed and paraffin embedded creating two matched sub-groups. To assess assay sensitivity one sample was diluted to eight different concentrations before fixing and embedding. The V4 and V6 regions of the 16s rRNA gene were amplified. Primer pairs created approximately 240bp and 98bp targets in E.coli respectively. PCR products were multiplexed and sequenced on an Illumina MiSeq. QIIME software was used for analysis.
Results: Analysis of alpha (within sample) diversity showed a significant difference between sub-groups when targeting V4 (p=0.05) but not V6. Analysis of beta (between sample) diversity showed a significant difference between sub-groups when targeting V4 (p=0.01) while the V6 region showed a reduced, but still significant (p=0.02) difference. The sensitivity assay showed comparable results down to 0.5% concentration levels.
Conclusion: To our knowledge this is the first feasibility study generating NGS data on the microbiome from FFPE faecal material. Variation between matched frozen and FFPE faecal material was less when targeting V6 compared to V4. We hypothesise this may be due to the shorter amplicon undergoing less DNA fragmentation in FFPE material.

Comparison of Mutational Calls Obtained with Pyrosequencing and the Affymetrix OncoScan® FFPE Assay in Patients with Colon Cancer Recruited to the NCRI FOxTROT Trial

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There are several platforms available for DNA mutation detection in formalin-fixed paraffin-embedded (FFPE) material, all with their relative strengths and weaknesses. We investigated the OncoScan® FFPE Assay Kit (OncoScan) in comparison to pyrosequencing in patients with operable colon cancer recruited to the phase II component of the NCRI FOxTROT trial of pre-operative vs. post-operative chemotherapy. FFPE samples of tumour from the resection specimens of 132 cases were tested for KRAS 12/13/61 and BRAF V600E mutations using pyrosequencing. The OncoScan assay allows for the interrogation of 74 mutations across nine genes. Pre-extracted DNA was analysed on the OncoScan assay and quality control (QC) scores generated, indicating confidence in mutation calling results. The mutational status of all samples was automatically assessed in the Affymetrix SM Viewer, and then manually confirmed. This work is presented on behalf of the FOxTROT Collaborative and was part funded by a PathSoc fellowship. Out of 132 samples, 22 failed OncoScan QC thresholds, however, only 10 of these were deemed inconclusive by manual interrogation. 130 samples were interpretable by pyrosequencing. Of the 120 samples that produced conclusive results on both platforms, the concordance rate was very high at 95.8% when calling a mutated versus non-mutated KRAS/BRAF status. Mutations were 'missed' by pyrosequencing in only 1 case (0.8%) and by OncoScan in 4 cases (3.4%). In addition, the OncoScan assay provides mutational data in additional genes along with copy number (CN) and loss of heterozygosity (LOH) information. In patients with colon cancer recruited to the NCRI FOxTROT trial, the OncoScan FFPE assay shows good correlation with pyrosequencing when determining the mutational status of KRAS/BRAF. Although pyrosequencing has a slightly lower failure rate, the OncoScan has the added advantage of targeting more mutations, producing genome wide CN, and LOH information in one assay.

Whole-Mount Microscopic Sections Reveal that Denonvilliers' Fascia is One Entity and Adherent to the Mesorectal Fascia: Should we still Question the Anterior Plane in TME?

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Excellent anatomical knowledge of the rectum and surrounding structures is essential for total mesorectal excision (TME). Denonvilliers' fascia (DVF) has been frequently studied, though the optimal anterior plane in TME is still disputed. The relationship of the lateral edge of DVF to the autonomic nerves is also unclear. We studied whole-mount microscopic sections of en-bloc cadaveric pelvic exenteration specimens and describe implications for TME. Four human adult cadaveric specimens (two males, two females) were obtained from the Leeds GIFT Research Tissue Programme. Paraffin-embedded mega-blocks were produced and serially sectioned at 50 and 250 µm intervals. Sections were stained with haematoxylin & eosin, Masson's trichrome and Millers' elastin. Additionally, a developmental series of eleven human fetal pelvic specimens (embryonic age of 9-20 weeks) were studied. DVF consisted of multiple fascial condensations of collagen and smooth muscle fibres and was indistinguishable from the anterior mesorectal fascia and the capsule of the prostate or posterior vaginal wall. The lateral edges of DVF appeared fan-shaped, and the most posterior part was continuous with the mesorectal fascia. Peri-rectal fasciae were not identified in fetal specimens. DVF is adherent to and continuous with the mesorectal fascia. Optimal surgical dissection during TME should be carried out anterior to DVF to ensure radical removal, particularly for anterior tumours. Autonomic nerves are at risk, but can be preserved by following the mesorectal fascia along the anterolateral mesorectum. The lack of evident fasciae in fetal specimens suggests that these might be formed in later developmental stages.

The Anatomy of the Perineal Body in Relation to Abdominoperineal Excision for Low Rectal Cancer

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The perineal body (PB) is poorly understood. In abdominoperineal excision (APE), there is no natural dissection plane through the PB. Knowledge of the PB is essential to avoid straying in to incorrect planes leading to tumour perforation and unnecessary urogenital and anorectal injuries. This study describes the anatomy of the PB and the implications for APE. Six human adult cadaveric specimens (three males, three females) were obtained from the Leeds GIFT Research Tissue Programme. Paraffin-embedded mega-blocks containing the PB were produced and serially sectioned at 50 and 250 µm intervals. Sections were stained to reveal collagen and elastin, and with an antibody against α-smooth muscle actin. The PB is formed of a fibromuscular mass, which was thicker and wider in female specimens compared to males, extending from the external anal sphincter to the rectogenital septum. Muscles from the urogenital diaphragm and anterior rectal wall anchored into the PB. The longitudinal muscle (LM) of the rectal muscularis propria extended in anterolateral directions and intertwined with the somatic pelvic floor muscles to create strong fixation of the anorectum. The LM plays a dominant role in the formation of the PB. Surgeons should be aware of the complex course of the LM through the PB to prevent injuries to the urogenital organs and perforation of the anterior rectal wall. The perineal phase of an APE starts with excellent exposure followed by proper tension on the PB to allow safe dissection through the densely-packed fibromuscular mass.

Impact of Tumour Budding in Oesophageal Adenocarcinomas

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Purpose of the study: Tumour budding has prognostic significance in many carcinomas and is defined as the presence of detached isolated single cells or small cell clusters (up to 5 isolated cells) scattered in the stroma. Tumour budding can be observed at the peripheral invasion front (peritumoural budding=PTB) and/or within the tumour (intratumoural budding=ITB). For oesophageal adenocarcinomas there are currently only few data about the impact of this morphological feature. In the present study we investigated PTB and ITB in a well characterized collective of primary resected oesophageal adenocarcinomas of two centers.
Methods: Whole tissue sections of 201 resection specimens were analyzed. Tumour buds were highlighted by pancytokeratin staining. PTB and ITB were scored across 10-high-power-fields (HPF). Results were correlated with clinico-pathological and follow-up data.
Summary of Results: Interobserver agreement between two independent investigators was substantial to excellent ($p < 0.001$, intraclass correlation coefficient = 0.77 for ITB and 0.92 for PTB). The median count of tumour buds was 130/10 HPF for PTB (range 2-593) and 80/10 HPF for ITB (range 1-656). PTB and ITB correlated significantly with each other ($r=0.9$; $p<0.001$). High PTB/ITB rates were associated with advanced tumour stages ($p<.001$ each), presence of lymph node metastases ($p<0.001/p=0.002$), worse tumour differentiation (grading; $p<0.001$ each) and higher rate of incomplete tumour resection ($p=0.003/p<0.001$). In addition, PTB was a significant predictor for the presence of lymph node metastases as well as tumour grading ($p>0.02$ each). Survival analysis showed a trend for an association with worse survival for both high grade PTB ($p=0.085$) and ITB ($p=0.082$), but pT and pN category were better predictors for survival ($p<0.001$ each).
Conclusions: Peripheral and intratumoural budding can be observed in oesophageal adenocarcinomas in various degrees. High grade budding is associated with aggressive tumour phenotype. Assessment of tumour budding may provide additional prognostic information about tumour behavior and may be useful in specific cases for better risk stratification of oesophageal adenocarcinoma patients.

WRN Promoter CpG Island Hypermethylation Does Not Predict a Favourable Outcome for Irinotecan-treated Metastatic Colorectal Cancer Patients

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Purpose: *WRN* promoter CpG island hypermethylation in colorectal cancer (CRC) has been associated with increased sensitivity to irinotecan-based therapies. We aimed to characterize methylation of the *WRN* promoter; determine the effect of methylation upon expression in CRC; and determine whether *WRN* methylation predicted the response to irinotecan-containing therapy in patients with advanced stage CRC.
Design: *WRN* methylation status was assessed using (quantitative) methylation-specific PCR and bisulphite sequencing assays. *WRN* expression was determined using qRT-PCR and Western blotting. *WRN* methylation status was correlated with overall survival (OS) and progression-free survival (PFS) in 183 patients with advanced CRC, where 90 received capecitabine monotherapy (CAP) and 93 received capecitabine plus irinotecan (CAPIRI) therapy as part of the CAIRO Phase III clinical trial.
Results: *WRN* mRNA and protein expression was low in colon cancer cell lines and in primary CRCs, and was largely independent of *WRN* methylation status. Patients with CRCs having methylated *WRN* had a shorter OS compared to patients who had cancers where *WRN* was unmethylated (hazard ratio [HR]=1.6 (95% CI 1.2-2.2), p=0.003). When looking at PFS, patients did not benefit from adding irinotecan to CAP when *WRN* was methylated (HR=1.1 (95%CI 0.69-1.77), p=0.7). In contrast, patients with unmethylated *WRN* showed a significantly longer PFS when treated with CAPIRI compared to CAP alone (HR=0.48 (95%CI 0.32-0.70), p=0.0001).
Conclusion: *WRN* promoter CpG island hypermethylation does not predict improved clinical outcomes of CRC treated with irinotecan-based therapy. *WRN* promoter methylation is independent of *WRN* expression in CRC.

EBV Positive Mucocutaneous Ulcer with Lymph Node Involvement Causing Sigmoid Stricture – A Case Report

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EBV Positive Mucocutaneous Ulcer (EBVMCU) is a relatively recently described EBV related disease with specific microscopic appearances, immunophenotype and usually good prognosis. Only a small number of cases presenting in the gastrointestinal tract have been described, most associated with iatrogenic immunosuppression. We present a case of a 74 year old woman with past history of breast cancer 6 years ago treated with wide local excision and radiotherapy, who presented with sigmoid stricture that was clinically considered to be due to diverticular disease requiring sigmoid colectomy. A deep ulcerating lesion was found at cut-up that was composed of granulation tissue and a dense infiltrate of lymphoid cells, plasma cells and macrophages with intermixed large atypical, Hodgkin/Reed-Sternberg (HRS)-like cells with the following immunophenotype: CD30+, LMP1+, EBER ISH+ and CD15-, CD20-. Some of the lymph nodes harvested from the specimen also showed a para-cortical infiltrate of small lymphocytes with eosinophils and occasional HRS-like cells. These morphologic and immunophenotypic features are considered to be compatible with EBVMCU with HRS-like cells most likely, in the absence of iatrogenic immunosuppression, secondary to age related immunosenescence. Conservative management was advised. Full staging investigations were performed which confirmed that the disease was confined to the gastrointestinal tract. She remained asymptomatic seven months after the initial diagnosis.

Genetic Mechanisms In Colorectal Polyposis

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Familial Adenomatous Polyposis (FAP), MUTYH-Associated Polyposis (MAP) and Polymerase Proofreading-Associated Polyposis (PPAP) are syndromes of adenomatous polyposis, and are due to mutations in APC, MUTYH, POLE and POLD1. Patients with multiple colorectal polyps undergo diagnostic analysis of APC/MUTYH and in 50-80% of cases, a mutation is identified. The aim of this study is to identify novel genetic mutations in patients with multiple polyps who have no mutation identified in the known disease-associated genes.
80 patients with ≥ 10 colorectal polyps have been recruited. All are negative for APC/MUTYH mutations following sequence/dosage analysis of coding exons, and patients have been screened for Pol mutations. Haloplex (Agilent) will be employed for sequence capture of the entire APC/MUTYH genes, followed by ultra-deep sequencing (UDS) on a HiSeq (Illumina). Samples will also undergo cDNA sequencing to screen for allelic imbalance and splicing abnormalities, and qPCR will be employed to assess gene expression. The characterisation of novel genetic variants associated with polyposis will allow more appropriate clinical management of patients. If the results of this study support the efficacy of this technique over current diagnostic protocols, improving the rate of mutation detection, then it may be readily translatable to clinical practice.
This project has been partly funded by a grant from the Pathological Society.

A 6-Years Review of Colorectal Cancer Resections Reporting as per Royal College Guidelines

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Objectives: (1) Are we following Royal College guidelines to report colorectal cancer resections and comparison with previous results? (2) To compare the frequencies of important prognostic factors to those of national standards.
Standards and Targets: (A) All the colorectal cancer resection reports should follow the minimum dataset by RCPath (MDS). (B) The frequency of important prognostic factors should be, mean number of lymph nodes examined at least 12, extramural venous invasion (EMVI) at least 25%, serosal involvement at least 20% for colonic cancers and 10% for rectal cancers.
Methodology: Retrospective done from Jan 2013-Dec 2013 (comparison with previous 5 years audit cycle). All cases of colorectal cancer resection included.
Results: (A) Compliance to Royal College minimum dataset. Clinical details 100%. Macroscopic description: Tumour site,size, distance to nearer cut end, relation to peritoneal reflection, plane of surgical excision 100%, tumour perforation 98%, and distance of tumour to dentate line in APR specimens 80%. Microscopic Description: Tumour type, differentiation, margin involvement, extent of local invasion, number of lymph nodes 100%, extramural venous invasion 99%, distance of tumour to NPRM 97%, response to neoadjuvant therapy 97%, histologicaly confirmed distant metastasis 94%, background abnormalities 94% and maximum distance beyond muscularispropria 92%. Pathological staging: complete resection at all margins, Dukes stage, pT 100%, pN 98% and pM 85%. (B) Frequency of prognostic indicators. Mean number of lymph nodes 24, frequency of EMVI 39%, frequency of serosal involvement in colonic cancers 34% and those of rectal cancers 12%.
Conclusion: In colorectal cancer, high-quality pathology reporting is vital. In general, all the important diagnostic criteria were included in the reports. 22 out of 24 core items were documented in above 90% of the cases. Our reporting frequency has been consisitently high above standard over 5 years

Assessment of Lymph Node Yield in Colorectal Cancer

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Purpose of the Study: The presence of lymph node metastasis is an important prognostic factor in colorectal cancer. Lymph node yield is a key parameter in the assessment of the quality of histopathology reporting of colorectal cancer excision specimens. The Royal College of Pathologists have set a standard of 12 for the median number of lymph nodes examined per specimen.
Methods: This study assesses the trend in lymph node yield from colorectal cancer excision specimens in a prospectively collected dataset compiled from the histopathology reports of 2646 patients who underwent surgery for primary colorectal cancer between 2005 and 2014 in a single institution.
Results: There was a significant increase in the total number of lymph nodes over the time period of this study from a mean of 14.91(median 14) in 2005 to a mean of 22.13 (median 21) in 2014, when all cases were assessed (n=2646). At least 12 lymph nodes were examined in 85% of cases. Over the same time period, in those cases where there had been no neoadjuvant therapy (n=2120), the mean number of lymph nodes examined rose from 15.36 (median 14) in 2005 to 22.44 (median 21) in 2014. In Dukes A cases the mean lymph node yield was 16.04 while Dukes' B cancers had a mean lymph node yield 19.48 and Dukes C cases had a mean lymph node yield of 18.42. Comparison of tumour site showed that proximal colon cancers had a mean lymph node yield of 19.46, distal colon cancers had a mean lymph node yield of 17.01 while rectal cancers had a mean lymph node yield of 19.6. The mean lymph node yield in cases following neoadjuvant therapy (n=526) increased from 12.03 in 2005 to 20.92 in 2014.
Conclusions: The data indicates that in this centre there is increasing lymph node yield from colorectal cancer excision specimens. The median number of lymph nodes examined surpasses the standard indicated by the Royal College of Pathologists.

The Relationship of Lymph Node Yield and Proportion of Dukes' C Colorectal Cancers

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Purpose of the Study: The presence of lymph node metastasis is an important prognostic factor in colorectal cancer. The Royal College of Pathologists' guidelines on reporting colorectal cancer indicates that all lymph nodes should be examined as it is expected that an increase in the number of nodes examined would result in an increase in the number of lymph node positive (Dukes' C) colorectal cancers.
Methods: This study compares the lymph node yield from colorectal cancer excision specimens and the proportion of lymph node positive cases over time in a prospectively collected dataset compiled from the histopathology reports of patients who underwent surgery for primary colorectal cancer between 2005 and 2014 (n=2120). Patients who had received neoadjuvant therapy were excluded from the study.
Results: There was a significant increase over time in the total lymph node yield per case, from a mean of 15.36 in 2005 to a mean of 22.44 in 2014 (Pearson correlation, r=0.939, p<0.001). No such trend was observed in the number of Dukes' C cases, with 44.3% of cases in 2005 lymph node positive and 43.2% of cases lymph node positive in 2014 (Pearson correlation, r=-0.268, p=0.454). When bowel screen detected cases are excluded the mean lymph node yield increased from 15.48 in 2005 to 22.9 in 2014 (Pearson correlation, r=0.940, p<0.001). There was no increase in the number of Dukes' C cancers, with 45.7% of cases in 2005 lymph node positive and 44.2% of cases lymph node positive in 2014 (Pearson correlation r=-0.188, p=0.604).
Conclusions: The data indicates that in this centre, there is an increasing yield of lymph nodes from colorectal cancer excision specimens, without necessarily a similar increase in the number of lymph node positive colorectal cancers even when the confounding effects of bowel screening and neoadjuvant therapy are excluded.

Audit of the Clinical Information Provided in Colorectal Histology Request Forms

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Background: By its very nature surgical pathology depends heavily on the input of clinicians and surgeons. The pathologist's need for adequate clinical information before diagnosis can be made has been highlighted in the past.
Aim: To audit the quality of clinical information provided in colorectal resection histology requests for the past 5 years. According to the Royal College of Pathologists' guidelines for reporting colorectal cancer histopatholy reports the following characteristics were examined: (1) the presence of a diagram of the surgical procedure, (2) if the cancer has been detected as part of the bowel cancer screening programme, (3) the histological type of tumour if known, (4) if there is history of inflammatory bowel disease or familial cancer, (5) the pre-operative stage of tumor, (6) whether or not pre-operative therapy has been given, when it finished and its nature, (7) if open, laparoscopic or robotic surgery has been performed, the type and dissection plane of the operation.
Methods: Data from 500 patients with large bowel resections were collected between 2/12/2009 and 18/11/2014.
Results: Out of the 498 histology request forms, only two had a diagram present (0.4%) and three (0.6%) reported that the tumour was detected in the bowel cancer screening programme. The histological type was reported in 72 out of 496 samples (14.52%) and the presence of IBD or familial cancer was reported in 2 out of 497(0.4%). The pre-operative stage of tumour was recorded in 27 out of 496 reports (5.4%) and the pre-operative therapy given in 56 out of 496(11.3%). Finally, the type of surgery and dissection were adequately documented in 221 out of 497 reports (44.5%).
Conclusions: Overall, the quality of reporting of clinical information in histology reports remains suboptimal. This audit identified important areas in which reporting quality needs to be improved.

An Audit of Duodenal Biopsies Submitted for Histopathological Assessment of Possible Coeliac Disease

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Purpose of the Study: We performed an audit to assess local compliance with guidelines published by the British Society of Gastroenterology in 2010 and 2014 that recommend a minimum of four duodenal biopsies for the histopathological assessment of possible coeliac disease. The 2014 guidelines also recommend biopsies from the duodenal bulb (D1).
Methods: The records of 2744 adult duodenal biopsies, received for histopathological assessment of possible coeliac disease between December 2013 and November 2014 were reviewed.
Summary of Results: Fewer than the recommended minimum number of biopsies were taken in 58% of cases. The precise sample site was unclear in 51%. The diagnosis was 'normal' in 84% of biopsies and supportive of coeliac disease in 5%. D1 was sampled in 93 (3%) cases, with D1 the only site sampled in 22 (<1%). When D1 was sampled with D2-D4 biopsies, the same diagnosis was made in each biopsy pair in 53% of cases (38/71), while a different diagnosis was made in the D1 biopsies in 37% (26/71) (e.g. gastric heterotopia/ metaplasia in 43%). In 10% of cases (7/71) the biopsies were reported together as one diagnosis. In no cases were D1 biopsies consistent with coeliac disease when accompanying D2 biopsies were normal. In three cases, features consistent with coeliac disease were present in the D2 biopsies but not the accompanying D1 biopsies.
Conclusions: Guidelines for adequate duodenal sampling are not always adhered to locally and the precise biopsy site is not always clearly stated. Endoscopic sampling of at least four biopsies, in line with existing and updated national guidelines, plus clear labelling of the site to aid accurate histopathological assessment of biopsies from different areas of the duodenum, is recommended. D1 sampling is rare in our institution; however, it has only recently been recommended in the updated 2014 guidelines, so this practice may increase in the future.

Does the Bowel Cancer Screening Program Detect Significant Upper Gastrointestinal Pathology?

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Purpose of Study: To determine whether Bowel Cancer Screening Programme (BCSP) patients with non-malignant lower gastrointestinal (LGI) pathology have subsequent upper gastrointestinal (UGI) pathology which may account for a positive faecal occult blood (FOB) test.
Methods: LGI specimens from 333 patients were submitted for histological examination as part of the local BCSP between May 2008 and April 2010. From the same patient cohort, subsequent gastrointestinal (GI) biopsy results from the following 3-5 years were recorded retrospectively and analysed, with particular focus on UGI pathology.
Summary of Results: 332 patients were included (233 male, 99 female), with median age of 66 years (range 60-78 years). 601 specimen results were reviewed, with most patients having more than 1 biopsy. Initial BCSP specimens included 50 adenocarcinomas (15% of patients), 411 adenomas (257 tubular, 145 tubulovillous and 9 villous) with a high grade dysplasia (HGD) rate of 5.1%, 136 hyperplastic polyps, 23 inflammatory changes, 26 other findings and 46 normal. Subsequent LGI biopsies from the same patient cohort contained 261 adenomas with a HGD rate of 3.8%. 28 of the initial 322 patients (who had non-malignant LGI results) had subsequent UGI biopsies (including oesophageal, gastric and duodenal). These UGI results included 2 adenocarcinomas, 2 H.pylori change, 1 coeliac disease, 3 chemical gastropathy and 20 normal/minor inflammation.
Conclusion: A high rate of colorectal adenocarcinoma and adenomas are detected through the BCSP. A very small number of patients (2 out of 332) also had UGI malignancy following a diagnosis of LGI adenomatous polyps. Both presented with widespread metastatic disease more than 6 months after initial BCSP investigation. These cases seem to be exceptional in the context of the BCSP, which is not designed to detect UGI pathology.

The Role of Ki67 Expression on Cellular Organisation Leading to Progression Towards Oesophageal Adenocarcinoma

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Introduction: Ki67 is a proliferation marker that is exclusively present in dividing cells and absent in resting cells. Its expression has already been studied in different cancers and used to understand the cellular organisation of Barrett's epithelium (Lavery 2014). However, very little is known about the cellular organisation based on Ki67 expression patterns in upper GI sequence. This study aims to examine the cellular organisation as defined by Ki67 expression patterns in upper GI cancer sequence.
Methods: Ki67 expression within Barrett's crypts was assessed in 40 cases (NBDE 11, LGD 15, HGD 14). The Barrett's crypts were divided into three equal regions: crypt base (bottom third), middle region and the surface (upper third), respectively. Ki67 was scored using the Allred system and analysed using one-way ANOVA with Bonferroni post-hoc analysis.
Results: One-way ANOVA showed significant difference across the three groups ($p < 0.0001$). Bonferroni post-hoc analysis showed significant difference in the surface architecture between NBDE and HGD ($p < 0.0001$) and LGD and HGD ($p < 0.0001$). For the middle region, although there was no statistical significance between the groups, NBDE and LGD and LGD and HGD showed statistical trends ($p = 0.079$ and $p = 0.089$ respectively). For the basal compartment there was significant difference between NBDE and LGD ($p = 0.035$).
Conclusion: This study showed for the first time a significant difference in the Ki67 expression between NBDE, LGD and HGD in the basal and surface regions. Middle compartments showed trends but additional NDBE, LGD and HGD groups need to be analysed to increase the statistical power. The results warrant further molecular analysis between the various groups and show a clear role for proliferation in the maintenance of the cellular architecture and organisation across the upper GI groups which might help in the understanding of the origin and development of OAC.

Audit of Compliance with Standards in the Royal College of Pathologists Dataset for Colorectal Cancer (July 2014, 3rd Edition)

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Purpose of the Study: Identify whether the department is compliant with the following national standards set by the Royal College of Pathologists in their revised dataset for colorectal cancer histopathology reports (July 2014, 3rd edition):
• Median number of lymph nodes examined should be greater than twelve.
• Frequency of serosal involvement should be at least 20% for colonic cancers and 10% for rectal cancers.
• Frequency of venous invasion should be at least 30%.
• 95% of reports must contain structured data.
• 80% of cases are authorised within seven calendar days of procedure, 90% within ten calendar days.
Method: A retrospective audit reviewing all colorectal adenocarcinoma cases seen in 2013 (identified by a laboratory computer system search).
Summary of Results:
Total of 139 resection cases identified (104 colonic and 35 rectal).
• Median number of lymph nodes examined is 20
• Frequency of serosal involvement is 23% for colonic cancers and 2.9% for rectal cancers.
• Frequency of venous invasion is 30.9%
• 100% of reports contain structured data
• 16.5% of cases were reported within seven calendar days, 66.2% within ten calendar days
Conclusions: The department is compliant with most standards, except:
• Frequency of serosal involvement in rectal cancers (suggested contributing factors for this include effect of pre-operative therapy, tumour regression and recent changes in surgical practice).
• Turnaround times: suggested contributing factors include increased departmental workload, retirements and reduced reporting capacity.
The following action plan was implemented to improve compliance with standards:
• Ensure all pathologists are aware of results via presentation/dissemination of audit report
• Identify issues affecting turn-around times and improvement strategies
• Support recruitment to increase reporting capacity.
• Maintain awareness of the need to recognise serosal involvement in rectal excisions.
• Re-audit in 1 year.

The role of IL-33/ST2 axis in Colorectal Cancer

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Background: ST2 and its ligand IL-33 are members of the TLR /IL-1 receptor family. Three isoforms of ST2 exist: a trans-membrane receptor (ST2L), a secreted soluble form (sST2), and a variant form (ST2V). Changes in IL-33 and ST2 have been reported in numerous cancers but their role in colorectal cancer (CRC) is unclear.
Purpose: To investigate the role of the IL-33/ST2 pathway in CRC.
Methods: ST2L and IL-33 mRNA levels were examined by qRT-PCR. sST2 and IL-33 serum levels were examined by ELISA. IL-33, ST2V and ST2L, were characterized by immunohistochemistry (IHC) on formalin-fixed paraffin-embedded CRC blocks.
Results: Expression levels of IL-33 and ST2L were examined in a cohort of 25 CRC cases. Levels of ST2L mRNA and protein were significantly reduced in CRC in comparison to adjacent non-tumour colonic mucosa as assessed by both qRT-PCR and IHC. Serum levels of IL-33 were also significantly reduced in this cohort in comparison to 15 healthy volunteers. These results were confirmed in a second patient cohort of 68 CRC cases analysed by IHC. ST2L showed decreased incidence and staining intensity with increasing TNM and pT stage. Neither ST2L nor IL-33 were over-expressed in colorectal metastasis relative to primary CRC. Following three year post-surgery analysis of cohort 2, cases with strong/moderate ST2L were associated with improved patients' survival in comparison to weak/negative cases. ST2V expression was unaltered in CRC relative to normal colon.
Conclusions: This data indicates a potential protective role for IL-33/ST2 in CRC.
This research has been awarded the small grant scheme by the Pathological Society of Great Britain & Ireland.

A Pilot Online Digital Archive of Colorectal Polyps and Teaching Cases for the Irish National Bowel Screening Programme

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Aim: The design and maintenance of a pilot online digital archive of archetype colorectal polyps and gastrointestinal (GI) teaching cases for the National Bowl Screening programme (BowelScreen) in the Republic of Ireland.
Methods: Suitable internal and referral cases were identified by BowelScreen consultants at Saint Vincent's University Hospital. These cases were subject to both internal and external review, by the Mater Misericordiae University Hospital, and represented typical examples of lesions seen in a National Bowel Cancer screening programmes (e.g adenomas, SSLs, adenomas with misplacement, TSAs). Representative slides, including immunohistochemistry, were anonymised and digitised using the Hamamatsu NanoZoomer Digital Pathology (NDP) whole slide scanner platform and associated software packages (NDP scan and view).
Whole slide images (WSI) were uploaded to secure cloud storage using a generic file transfer protocol program. WSI were collated into 18 cases and accessible via the PathXL gateway (pathXL.co.uk) by approved users via an online case referral and reporting system. Users were notified of pending cases via email and the viewing of WSI occurred within the user's web browser utilising an online version of NDP view program and did not require local use of propriety software. Each case was referred across the two participating sites and scored in four areas; diagnosis concordance, quality of WSI, web interface and the online referral and reporting system.
Conclusion: With the maturation of technology involved in digital microscopy a digital archive program is now a feasible approach to the standardisation of diagnosis and a useful adjunct to traditional optical microscopy in education within the National Bowel Screening programme.

Diffuse Ganglioneuromatosis of the Gut: A Rare Case Report

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Introduction: Intestinal ganglioneuromatosis is a rare pathological condition of the enteric nervous system and involves proliferation of ganglion cells, Schwann cells and nerve fibres in the bowel wall Diffuse ganglioneuromatosis is rare in adults.
Case presentation: We are presenting an extremely rare case in a 48 years male with history of neurofibromatosis presenting with gripping abdominal pain. On colonoscopy, there were multiple polyps in the intestine with abnormal proximal ileum. On macroscopic examination, the bowel showed various nodular lesions measuring 1cm. On microscopy,there was transmural infiltration of diffuse spindle cells with fibromyxoid areas and had ganglion cells.The tumour cells were positive for S100, and focally for CD34. Alk and CD1a were negative. There were no features of malignancy.
Conclusion: In adult patients presenting with obstructive gastrointestinal symptoms, the rare possibility of ganglioneuromatosis should be considered and investigated in order to avoid unnecessary and extensive surgical intervention.

A Trainee's Perspective of the Biomedical Scientist (BMS) Histopathology Reporting Pilot

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Purpose of the Study: To see if it is feasible to train BMS staff to undertake independent reporting and cut-up of gastrointestinal and gynaecological histopathology cases.
Methods: This three year training programme commenced in 2012 and uses the training of junior medical staff in histopathology as a template. A portfolio must be completed each year. The minimum components of the portfolio are evidence of cases dissected and reported, eighteen workplace based assessments, an audit and an educational case report. Trainees must pass an Objective Structured Pathology Examination (OSPE) at the end of Year One. Year Two is entirely portfolio based. Trainees must sit an Exit Examination at the end of Year Three.
Summary of Results: Five candidates from the first intake sat and passed the OSPE in 2013. The same five candidates are currently in Year Three and will sit the Exit Examination in September 2015.
Conclusions: A committed and conscientious BMS can learn how to report histopathology cases. However, if this is to be achieved, the department in which he or she works must also be committed and supportive.

Carcinoid Tumour of the Appendix: A Case Report

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A case of a 24 years male operated on for acute appendicitis and an incidental finding of a carcinoid tumour at the tip is reported. The tumour was less than 2 cm in greatest dimension but it infiltrates through the wall of the appendix into the surrounding fat. It stains positive for the neuroendocrine markers. Carcinoid tumour of the appendix is unusual, but it has to be looked for during examination of appendectomy specimens done for appendicitis (0.5%). Women are more frequently affected than men (3:1) and the tumour is usually small less than 1 cm in diameter and frequently located at the tip. It is usually diagnosed incidentally after an operation for acute appendicitis and sometimes during other procedures (colectomy, cholecystectomy and others). The tumour rarely metastasis to the liver and this is usually related to the tumour diameter) and can cause a "carcinoid syndrome": flush, diarrhea bronchoconstriction, cardiac valve disease. Diagnosis is made by the pathologist and staging by conventional radiologic procedures (TAC, US), dosage of neuroendocrine mediators such as 24 hours urinary 5-HIAA. Simple appendectomy is adequate treatment for appendicular carcinoids less than 1 cm in diameter. Adequate treatment for tumours greater than 2 cm is right hemicolectomy. The mangement of tumours 1 to 2 cm range is controverisal, but generally, appendectomy alone is sufficient except when meso-appendix is invaded. Carcinoid tumour of the appendix has a good prognosis with a 5-year-survival rate, of 85-100%.

The Prevalence of Epithelial Changes in Helicobacter Pylori-Associated Gastritis in Oman: A Retrospective Study

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There is Strong association between H. pylori gastric infection and epithelial changes and progression to cancer. It has been shown that H pylori infection is strongly associated with high proliferative activity and it could be a risk of initial step of gastric carcinogenesis. The aim of this study was to examine the association between epithelial changes in the gastric mucosa and gastric H pylori infection in Oman by retrospective examination of the gastric biopsies for patients presented to Sultan Qaboos University hospital (SQUH) in 2013. A total of 697 biopsies were studied with a prevalence of H pylori infection in 34% with about 13% showing epithelial changes, mainly intestinal metaplasia in 10% out of the H pylori positive cases, a few cases with low grade dysplasia and reactive atypia. In cocnlusion intestinal metaplasia was the main epithelial change that was related to H pylori infection. Further studues are required to investigate the relation between H pylori infection and the progression to gastric carcinoma.

The Effect of Changing Surgical Technique on Oncological Outcomes for Abdomino-Perineal Excision of Rectum in a DGH Setting

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Purpose of the study: Low rectal carcinoma may require abdomino-perineal excision of the rectum (APER), which has been associated with higher rates of tumour perforation and circumferential margin (CRM) involvement than anterior resection. This increases the risk of local recurrence and may necessitate adjuvant treatment. The Extralevator Abdominoperineal Excision of Rectum (eLAPE) in the prone position has been found to improve these outcomes and has been encouraged by the Low Rectal Cancer National Development Programme (LOREC). We aimed to assess the effect of increasing the practice of eLAPE on the histological and oncological outcomes in these cases in the Mid-Yorkshire NHS trust, a large District General Hospital.
Methods: In 2011 the number of surgeons routinely performing APER was reduced and all those performing the procedure had been trained in the cylindrical resection technique. Joint operating and laparoscopic procedures were encouraged. A retrospective review of case notes and histological reports between 2009 and 2012 was performed (before and after sub-specialisation). Patient demographics, histological findings and complications including local recurrence were recorded.
Summary of Results: Between 2009 and 2011, 39 APERs were performed, with tumour perforation in 5 (13%) and CRM involvement in 8 (21%) of cases. After sub-specialisation, 28 were performed. None were perforated and 2 cases (7%) showed margin involvement. Local recurrence occurred in two cases before specialisation and none after 2011 at the time of follow-up. Joint operating and subspecialisation increased the number of cases performed by each surgeon, and the number performed laparoscopically.
Conclusions: eLAPE in conjunction with departmental restructuring significantly improves immediate oncological outcomes in a DGH setting, with no effect on 30 day mortality. The technique may reduce local recurrence, although longer follow-up would be required.

- ***This work has previously been published as an abstract to the International Journal of Surgery 11 (2013) 589e685, under the title "Reducing Perforation Rates and Local Recurrence in Aper's in a DGH Setting". It has not been presented in a pathological conference or published in full previously.***

Systems Biology Approaches to Cervical Pre-Cancer Diagnostics

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Background: Systems biology uses computational and simulation approaches to interrogate gene expression datasets and explore biological pathways. By employing systems biology and data mining tools we can identify new biomarkers. Our objective was to ascertain the utility of a novel panel of systems biology derived biomarkers in cervical pre-cancer for more accurate grading and stratification of CIN disease.
Methods: This project is conducted within the framework of an FP7 funded programme "SYSTEMCERV". Gene pathways were analysed using MATLAB and SIRENE. Along with accessing KEGGS online database for gene prediction and DAVID for gene functional classification, we identified a novel panel of biomarkers. Gephi software was used to visualise communities of genes related to cervical pre-cancer and cancer progression. Clinical validation was performed by immunohistochemistry on a range of cervical LLETZ specimens (Normal, CIN1, CIN2 and CIN3). All patients gave written informed consent. In parallel, p16 IHC was performed on all specimens as a benchmark stain.
Result: The biomarker panel included TP63, epiregulin and desmoglein-3. Biomarker expression patterns were evaluated on 113 clinical samples, normal [n=13], CIN1 [n=32], CIN2 [n=34] and CIN3 [n=31]. Altered expression patterns were identified in CIN lesions as compared to normal cases. Desmoglein-3 showed significant difference in expression across different grades of CIN. Epiregulin played a suggestive role in identifying virally infected cells. TP63 showed the strongest correlation to p16. Expression increased with disease progression, indicating the capability of TP63 to demarcate between CIN 1, 2 and 3.
Conclusion: Novel biomarkers have the potential to distinguish between different grades of CIN based on protein expression status. This systems biology-based approach for identifying novel markers within gene pathways may significantly improve grading of CIN.

Defining the Raman Spectroscopic Signature Associated with Low and High Grade Cervical Disease

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The mortality associated with cervical cancer can be reduced if the disease is detected at the early stages of development or at the pre-malignant stage. The Pap smear is the current screening method, but is highly subjective and can often exhibit low specificity and sensitivity. For this reason, either a replacement or supportive technique is necessary to improve the quality of cervical cancer screening. Raman spectroscopy is a powerful tool that can generate a biochemical fingerprint of a sample in a rapid and non-destructive manner. In this study, Raman spectroscopy has been applied to the investigation of cervical cells from PreservCyt specimens. Raman measurements were taken from the nuclei of cervical cells from normal, CIN1, and CIN3 samples. These spectra were processed, analysed and used to define a spectral signature for each grade of cervical disease. Principal Component Analysis (PCA) was used to discriminate between the two data sets. Distinct Raman spectral differences were detected between normal, CIN1 and CIN3 cells. Notably, it was possible to observe spectral peak shifts representing fluctuations in Guanine (DNA/RNA), CH deformation in proteins and carbohydrates, Carbon-carbon double bonds in Phenylalanine, Tyrosine and Tryptophan, and Amide I. The PCA showed an excellent discrimination between the data sets. This study has shown that Raman spectroscopy can detect subtle changes between cervical cells, and may be a powerful tool for improved diagnosis of cervical dysplasia.

The Role of the TLR4 Pathway and the Spindle Assembly Checkpoint in Ovarian Cancer Prognosis

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Background: MyD88 and MAD2 are two potential prognostic biomarkers that have been investigated in ovarian cancer. High MyD88 and Low MAD2 IHC staining is associated with reduced PFS, both markers are also linked to paclitaxel chemoresistance.
Objectives: The main objective of this study was to assess the *in vitro* relationship between MAD2 and MyD88, through alteration of MAD2, MyD88 or its receptor TLR4 in two ovarian cancer cell lines using siRNA targeting MAD2, TLR4 or MyD88 and a MyD88 overexpression plasmid vector. Following overexpression/siRNA knockdown procedures, MyD88, TLR4 and MAD2 expression was assessed through qPCR and Western Blot analysis. Mir-433, Mir-21 and Mir-146a gene expression was also assessed by qPCR. Furthermore the effect of TLR4/MyD88 knockdown on chemoresponse was assessed in SKOV-3 cells using the CCK-8 assay.
Results/Discussion: It was found that knockdown or overexpression of MyD88 in SKOV-3 or A2780 cells respectively or knockdown of TLR4 in SKOV-3 cells had no effect on MAD2 expression or the expression of Mir-21, Mir-433 and Mir-146a. Interestingly however knockdown of MAD2 in both cell lines induced a 3 fold increase in TLR4 expression, furthermore knockdown of TLR4 in SKOV-3 cells was shown to restore chemosensitivity to paclitaxel.
Conclusion: The results demonstrate a potential *in vitro* link between TLR4 and MAD2 and support a role for TLR4 in paclitaxel chemoresistance.

The Complexity of Ovarian Cancer Resistance Mechanisms: A Novel, Clinically Relevant, *in-vitro* Investigation

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Ovarian cancer (OC) is the leading cause of death from a gynaecological malignancy. Standard chemotherapy often fails and patients relapse with chemoresistant disease. Novel carboplatin and taxol resistant cell lines were developed from UPN251 OC cells in a clinically relevant selection strategy to better understand resistant mechanisms in OC. UPN251-7C models carboplatin resistance and UPN251-7T models taxol resistance. UPN251-6CALT and UPN251-6TALT were exposed to alternating treatments of both agents during development. Affymetrix arrays were used to characterise gene/miRNA signatures linked with the development of chemoresistance in OC cell lines UPN251-7C and UPN251-7T. Bioconductor software, DAVID v6.7 and miRNA-target interactions (MTIs) analysis was carried out to identify de-regulated genes/miRNAs, gene pathways and gene/miRNA interactions involved in resistance. UPN251 sublines developed using taxol were significantly resistant to taxol, vinblastine and olaparib (P-gp substrates), and reversible with elacridar (P-gp inhibitor) treatment. Significant up-regulation ABCB1 was seen in UPN251-7T which was reflected at the protein level. SRPX2 was highly up-regulated in UPN251-7T. GLI3 and CCL20 were up/down-regulated respectively in UPN251-7C. GLI3 had a validated interaction with miR-205 down-regulated in UPN251-7C. LIN28B was highly deregulated in UPN251-7C and UPN251-7T and had a validated interaction with let-7i, down-regulated in UPN251-7C. P-gp over-expression is a dominant mechanism for taxol resistance in our cell lines. Mechanisms for carboplatin resistance are more complicated. The top deregulated genes are involved in numerous pathways including apoptosis, cellular transformation, signal transduction, and cell migration. Bioinformatics analysis and literature review identify LIN28B, GLI3, CCL20 and SRPX2 and miRNAs let-7i and miR-205 as strong potential biomarkers for carboplatin/taxol resistance in OC.

Cervical Glandular Neoplasia: The Influence of Excision Procedure on Margin Status

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The incidence of cervical glandular lesions is increasing. As these lesions frequently occur in women of reproductive age, the choice of treatment can impact on future fertility. Although cold knife cones (CKC) have been traditionally advocated for treatment of adenocarcinoma in situ (AIS), large loop excisions of the transformation zone (LLETZ) are increasingly used. We analysed excisions from 111 patients where AIS was confirmed prior to procedure over 5 years to assess the influence of excision procedure on final margin status. We tabulated whether margins were involved, close (lesional tissue less than 5mm from margin) or excised.

Results: LLETZs were performed in 70% (78 of 111 patients), CKC excision in 30% (33 of 111). Women who had LLETZs were younger than those having CKC excision (32.8 years versus 35.4 years). Positive margins were present in 21% (16 of 78) and close margins in 27% (21 of 78) LLETZ cases. For CKC, margins were positive in 9% of cases (3 of 33) and close in 6% (2 of 33). 28% of patients who had an initial LLETZ required a second procedure (11 patients had a further LLETZ while 11 had CKC excision).

Conclusion: Although complete excision is more frequently observed when CKC is performed, compared to LLETZ, for the treatment of AIS, CKC can impact on future pregnancies. Any cervical intervention must be cognisant of the sometimes conflicting needs of cervical preservation and the need for excision of a pre-malignant lesion.

The Role of MAD2 and MyD88 as Prognostic Indicators in Ovarian Cancer

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Background: The prognosis of epithelial ovarian cancer is poor in part due to the high frequency of chemoresistance. Recent evidence points to the Toll-like receptor-4 (TLR4), and particularly its adaptor protein MyD88, as one potential mediator of this resistance. Downregulation of MAD2, a key component of the spindle assembly checkpoint complex, has also been linked with paclitaxel resistance . Both markers have individually been shown to be associated with poor outcome in ovarian cancer. High MyD88 and low MAD2 immunohistochemical staining is associated with reduced progression free survival. The main objective of this study was to assess the combined utility of MAD2 and MyD88 in predicting patient prognosis.

Methods: Two tissue microarrays composed of cores from 51 high grade serous epithelial ovarian cancers patients were constructed and stained for MAD2 and MyD88. Staining was scored based on previously derived scoring schemes for MyD88 or MAD2 staining. The mean overall score from triplicate cores was then used to classify patients into high and low staining categories.

Results: A trend towards reduced progression free and overall survival was observed in patients with high myd88 and low mad2 expression.

Conclusion: The results demonstrate the combined utility of MAD2 and MyD88 as predictors of prognosis in ovarian cancer.

Identification of the Chemoresistant Component of a Novel Ovarian Cancer Stem Cell Hierarchy

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Purpose: Ovarian cancer is characterised by high rates of terminal, chemoresistant recurrence. Although chemoresistance is known to be a property of Cancer Stem Cells (CSCs), the mechanism is poorly understood. We have previously identified a novel four-member CSC Stem-Progenitor cell hierarchy for ovarian cancer. The aim of this study was to characterise the contribution of each member of the ovarian CSC hierarchy to chemoresistance.

Methods: The CSC hierarchy was assessed for tolerance to chemotherapy drug cisplatin (MTT assay) both as components of the parent population (A2780 cell line) and as isolated cell types. The hierarchy was additionally assessed in the long-term cisplatin-adapted 'A2780cis' cell line. Cell types were analysed and isolated via flow cytometry and assessed for stem cell characteristic via single-cell asymmetric division and murine xenograft tumourigenicity assays, and molecularly characterised (whole transcriptome arrays).

Results: Cisplain dose-response assays from A2780-derived CSC sub-populations indicated that only one of the four populations within the hierarchy had a high cisplatin-tolerance (IC50=10uM) compared to the other populations (IC50=4uM). This was notable as the relative cisplatin IC50s for the A2780 and A2780cis parent cell lines are 4uM and 11uM respectively. Treatment of the parent A2780 cell line with the IC50 (48 hours) resulted in a proportional 50% loss in each of the four cell types, suggesting that this specific CSC sub-population adapts to cisplatin over a longer period of time.

Conclusion: Although CSCs are known to be chemoresistant, the mechanism though which this is achieved is poorly understood. Our data indicates that only some members of a CSC hierarchy are responsible for chemoresistance. Notably, this sub-population appears to possess inherent chemoresistance in pre-treatmentcells. As such, it should be possible to target these CSCs in the primary malignancy to prevent chemoresistant recurrence.

Mixed Sex Cord Stromal Tumour of Ovary Containing Pseudoendometrioid Sertoli-Leydig Cell Tumour: a Rare Case Report

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Mixed sex cord-stromal tumours of the ovary are very rare. We report a case of mixed sex cord -stromal tumour (also referred to as gynandroblastoma) containing both Sertoli-Leydig cell tumour and adult granulosa cell tumour in a female 61 years old who presented with postmenopausal bleeding. On histology, the majority of the tumour represented an unsual form of well differentiated Sertoli-Leydig cell tumour with a pseudoendometrioid appearance. Minor foci of classic adult granulosa cell tumour were present. On immunohistochemistry, the tumour was diffusely positive for inhibin and SF1 and focally for calretinin, ER and CD56. EMA, PAX8 and CK7 were negative. As far as we are aware, this is the first report of an ovarian mixed sex cord-stromal tumour containing a component of pseudoendometrioid Sertoli-Leydig cell tumour.

Hypertrophic Herpes Simplex Simulating Vulval Cancer in an HIV Positive Patient: Case Report and Literature Review

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A 37 year old female patient presented with bilateral painful warty lesions on the labia majora. The patient had had HIV for a long time and was on highly active antiretroviral therapy. She also suffered chronic renal failure requiring haemodialysis three times weekly. Clinically, the lesions were highly suspicious of vulval cancer. The lesions increased significantly in size over a short period of time (2 months) requiring surgical resection under general anaesthesia. Histological examinations revealed polypoid lesions with prominent pseudoepitheliomatous hyperplasia and dense inflammatory infiltrate, composed mainly of lymphocytes and plasma cells, extending to the hypodermis. Numerous abscesses with large numbers of eosinophils were present withinin the hyperplastic epithelium. The typical intranuclear inclusions of herpes simplex virus (HSV) were identified. HSV immunohistochemistry was positive. This is a rare case of vulval HSV warts mimicking cancer. Oral acyclovir was administered following surgery and resulted in good control. Literature review shows only 6 previously described cases of verrucous HSV, Types 1 and 2, simulating neoplasia in patients with AIDS on antiretroviral therapy.

The Demographics of Uterine Cancers – A Teaching Hospital Perspective

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Purpose of the study: The demographics of endometrial cancer have evolved over recent years. These factors pose management dilemmas for women deciding to delay their fertility. We analyse premenopausal and postmenopausal women with endometrial cancer with a view to documenting the local aetiological and demographic factors that influence both the management and survival.
Method: We retrieved 280 sequential uterine cancers from our pathology database between July 2010 to June 2012. All relevant pathology, imaging and case notes were reviewed and the data analysed. The cut-off age for premenopausal to postmenopausal was taken as 52 years and the endometrial cancer subtypes(endometroid, serous, clear cell and carcinosarcoma) were documented for both groups together with history of other malignancies, recurrence and survival.
Summary of Results: In the premenopausal (PRM) group 77% had type I cancers and 23% had type II cancers. In the Postmenopausal(PM) age group 55% had type I cancers and 45% were type II. 77% had stage 1A disease in PRB vs. 17% in PM. Recurrence was 18% for type I and 20% type II for PRM vs. 9% for type I and 40% for type II in PM. 9% of PRM women had carcinosarcoma vs. 8% in PM group. No PRM patients with carcinosarcoma had a previous diagnosis of other malignancies however 29% of PM group with carcinosarcoma had breast cancer of which 100% were taking tamoxifen.
Conclusions: The two major subgroups showed a mark distinction in the incidence of the type I and type II cancers. Furthermore, we found 29% with carcinosarcoma in the postmenopausal group had a previous history of breast cancer. When these patients were matched to age and stage matched controls the survival was poorer.

Should We Follow-Up Endometrial Polyps? We Present a Teaching Hospital's Experience

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Purpose of the Study: The follow-up of endometrial polyps poses a clinical dilemma; no guidelines currently exist. Risk factors are age, obesity, hypertension and tamoxifen use. Patients may be asymptomatic or present with abnormal uterine bleeding. The literature contains proposals for follow-up but none have been adopted. Risk of malignancy is considered low (0-12.9%). We audited current follow-up practice within a teaching hospital with histological outcome.
Methods: Retrospective analysis of 4 years of endometrial curettings from a Trust histopathology database identified 302 patients with polyps. Polyps with hyperplasia were followed-up over 12-16 years. Divided into pre- and post-menopausal groups.
Summary of Results: 47 of 302 patients with polyps had hyperplasia: 6 atypical, 28 complex, 12 simple and 1 unclassified. 1 polyp contained adenocarcinoma at presentation. Only 26 patients with hyperplasia were followed-up. 10 patients underwent hysterectomy within the follow-up period. Of 10 hysterectomy specimens; 2 had carcinoma, 6 had hyperplasia (2 with and 4 without atypia), 2 had no endometrial pathology. 16 of the 47 cases with hyperplasia followed-up with pipelle/curettage monitoring: 13 cases had no endometrial hyperplasia or neoplasia, 1 complex hyperplasia without atypia and 2 were inadequate. Of the 6 patients with atypical hyperplasia, 2 regressed within 3 years.
Conclusions: 2 cases progressed to carcinoma. One symptomatic and post-menopausal, the other had HNPCC. Progression time was 5-18 months. We are in agreement with literature stating regression can occur in hyperplasia. Follow-up of polyps with hyperplasia should be confined to symptomatic post-menopausal patients and those identified as high risk.

Primary Mucinous Eccrine Adenocarcinoma of the Vulva – A Rare Malignant Adnexal Neoplasm at an Unusual Site

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Primary mucinous eccrine adenocarcinoma of the skin is a rare adnexal neoplasm, typically involving the head and neck region in the elderly population. Here we present a case of primary mucinous eccrine adenocarcinoma of the vulva; occurrence at this site is extremely rare, with only five cases published in English literature. A 62 year old female presented with a 10mm vulval lesion, clinically suspected to be an inclusion cyst. The lesion was removed and sent for histopathological assessment. Histological examination revealed a well circumscribed, partly encapsulated tumour composed of rounded and irregular nests of polygonal epithelial cells with scattered lumina, suspended in pools of extracellular mucin. The epithelial cell nuclei displayed a uniform chromatin pattern with small distinct nucleoli. The mucin pools stained positive for Alcian blue and dPAS. Immunohistochemical staining demonstrated positivity for CEA, CK7, GCDFP, oestrogen receptor, progesterone receptor, synaptophysin and chromogranin. Immunostaining was negative for CK20, CDX2, CA-125, TTF-1, CH5/6, CK14, HER-2, WT-1, CD56 and S100. Ki-67 proliferation fraction was approximately 5%. Overall, the findings were those of a mucinous eccrine adenocarcinoma with neuroendocrine differentiation. Following multidisciplinary discussion, and negative imaging of the breasts and gastrointestinal tract, a diagnosis of primary mucinous eccrine adenocarcinoma of the vulva was reached. Only a handful of cases of primary mucinous eccrine adenocarcinoma of the vulva have been reported. Metastatic disease, particularly from breast and colon, must be excluded. Follow up data from patients with primary mucinous eccrine adenocarcinoma of the skin suggests a high local recurrence rate (29.4%), necessitating close follow-up. However, risk of metastasis is low (9.6%).

A Rare Case of Vulval Myxoid Chondrosarcoma

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Introduction: Primary Extraskeletal Myxoid Chondrosaroma (EMC) of the vulva is a rare mesenchymal neoplasm. The myxoid tumour differential diagnosis on a core biopsy can be quite challinging. To date, few cases have been reported in the literature.
Case Report: A 42-year old woman noticed a swelling on the right side of the labia, thought to be a Bartholin's cyst in 2011. She was managed conservatively. She had drainage and marsupialization under general anaesthesia. This resulted in extreme bruising of the vulva. This was managed with antibiotics and non-steroidal anti-inflammatory medication, and it resolved after 3 weeks. Six months later, the patient presented again with a persistent vulval mass. A biopsy was obtained under general anaesthesia, and it showed a myxoid tumour with differential diagnosis of low grade chondroid tumour. An MRI was performed to assess the extent of the disease. The tumour was excised. At surgery, a 7 x 5 cm lobulated, extremely vascular vulval tumour was found. The tumour was inseparable from the inferior pubic ramus of the pelvic bone. A complete macroscopic resection was obtained. Histology confirmed low grade myxoid chondrosarcoma.
Conculsion: Vulval lesions with unusual characteristics or insidious evolution in the labia majora or Bartholin's glands area should be carefully and promptly investigated. Differential diagnosis of myxoid tumours in the vulva should include myxoid chondrosarcoma amongst other diagnoses.

An Investigation Into the Prevalence of Transformation Within a Cohort of Diffuse Large B Cell Lymphoma Patients

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Diffuse Large B Cell Lymphoma (DLBCL) is the most common high grade lymphoma in the UK. A proportion of DLBCL occur as the result transformation of a pre-existing low-grade lymphoma. We have analysed a cohort of 265 patients diagnosed and treated in Bristol for DLBCL over a 5-year period. 47 patients were considered transformed from a pre/co-existing low-grade component. 31 (66.0%) patients had documented evidence of low-grade lymphoma in the previous or in the same biopsy. Of these 31 cases, 13 arose from Follicular Lymphoma, 13 from Marginal Zone Lymphoma, 3 from Chronic Lymphocytic Lymphoma, and 1 from Lymphoplasmacytic Lymphoma. In 16 (34.0%) patients, discordant low-grade lymphoma was identified in the bone marrow during staging investigations. 4 were classified as Follicular Lymphoma, 2 Marginal Zone Lymphoma, 2 Chronic Lymphocytic Lymphoma, 1 Lymphoplasmacytic Lymphoma, 1 Non CLL -like Monoclonal B-cell Lymphocytosis. Five had an accompanying a low-grade component that could not be classified. None of the transformed high-grade lymphoma cases were EBV positive. In our cohort, we did not observe statistically significant difference in survival between the Transformed and Non-Transformed cases. An equal proportion of cases transformed from Follicular Lymphoma and Marginal Zone Lymphoma. 19.1% of Transformed patients had a previous history of another cancer, compared to 12.1% of Non-Transformed cases. Age, gender and a history of autoimmune disease were not associated with transformation. Transformed Chronic Lymphocytic Lymphoma, although rare, was as described highly aggressive. This study provides further information about nature of DLBCL with evidence of an associated low grade component.

An Investigation Into the Presentation and Nature of Diffuse Large B Cell Lymphoma Within a Large Patient Cohort

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Diffuse Large B Cell Lymphoma (DLBCL) is a high-grade cancer, accounting for approximately one third of lymphoma cases in the UK. DLBCL is an umbrella term, encompassing multiple distinct disease entities. We have studied a cohort of 265 DLBCL patients, diagnosed and treated in Bristol between 2009 and 2014. Clinical, epidemiological and histological data on each patient was complied into purpose-built database. The age at presentation ranged from 23 to 96 years, with a mean age at 68 years. 121 patients were female and 144 male. Disease was nodal in 43.8% and extranodal 56.2%. The most common extranodal sites were the GI tract (11.3%), skin (6.8%), CNS (4.2%), bone (4.2%) and ocular (2.3%). All DLBCL subtypes were observed. DLBCL, NOS was the commonest (87.9%), T Cell/Histoiocyte-Rich Large B Cell Lymphoma (4.2%) was the next most common subtype, followed by Primary DLBCL of the CNS (3.0%). A modified R-IPI (the patient performance score was unknown) was used to stratify the patients into four risk groups and was found to be predictive of patient outcome. The average LDH level was 733.5 IU/L, well above 480, the upper limit of the normal range. Of the cohort of patients, 52.8% achieved remission, 15.5% were alive with disease at the end of the study and 31.7% are now deceased. The majority of patients that did die did so within a year of diagnosis. In addition, 27 Bcl-2 negative patients were identified, with a mean age at diagnosis of 66.8 years. 11 of these patients were female and 16 male. The mean LDH level was 747.5 IU/L. 51.9% of the Bcl-2 negative cases are in remission, 25.9% are alive with disease and 22.2% are deceased, none of these proportions are significantly different from the cohort as a whole.

Composite Lymphoma of Diffuse Large B Cell Lymphoma with Hairy Cell Leukemia

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We have recently encountered an unusual case of composite lymphoma in a 63 year old gentleman. He presented with abdominal, retroperitoneal and mediastinal lymphadenopathy and enlarged spleen. Based on the core biopsy of a retroperitoneal lymph node, the diagnosis of diffuse large B cell lymphoma was made. A subsequent staging bone marrow however, revealed diffuse and heavy infiltration by hairy cell leukemia (HCL). Molecular genetic analysis of the bone marrow sample confirmed the BRAF c.1799T>A; p.Val600Glu (V600E) mutation commonly associated with HCL. Retrospective analysis of the retroperitoneal lymph node core biopsy revealed two populations of lymphoid cells. In addition to the dominant diffuse large cell component, there was a small population of small lymphoid cells showing hairy cell phenotype. The diffuse large B cell component was negative with hairy cell markers. Molecular analysis confirmed the BRAF mutation. Fluorescence in situ hybridisation (FISH) showed the presence of a BCL2 rearrangement in a proportion of cells. Therefore this case is best regarded as a composite lymphoma of diffuse large B cell lymphoma with hairy cell leukemia rather than blastic transformation of hairy cell leukemia. To the best of our knowledge, simultaneous occurrence of diffuse large B cell lymphoma and hairy cell leukemia in a lymph node has not yet been reported in the literature.

Bone Marrow Aspirates and Trephine Biopsies: An Audit of the Effects of Integrated Haematopathology Reporting

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Bone marrow examination by aspirate and trephine biopsy is an important haematological investigation. Ideally, aspirate findings should inform examination of the trephine biopsy, but if the two modalities are separate the aspirate report can be delayed and histopathologists may assess the trephine biopsy without being aware of the aspirate findings. We audited the availability of aspirate results to the histopathologist examining trephine biopsies, over the period in which our department implemented an integrated haematopathology reporting system. The effects on diagnostic concordance, turnaround times and immunohistochemistry requesting were also assessed. The setting was a regional specialist haematopathology centre. Prior to integration, a prospective audit of 101 consecutive trephine biopsies received by a senior haematopathologist was carried out, against standards set by the International Committee for Standardisation in Haematology. Data were collected from hospital computer systems. The move to integrated reporting involved the installation of new software (HiLIS) to specifically handle integrated haematopathology data. Ten months later, a retrospective analysis of a further 100 cases was performed using HiLIS data. Prior to integration, 35% of aspirates were reported within 3 days, and access to the aspirate report was available at time of examination for 61% of trephine biopsies. After integration, 96% of aspirates were reported within 3 days, and reports were available at time of examination for 100% of trephine biopsies. Diagnostic concordance was 72% initially, and 100% after integration. The mean number of immunostains requested per case was unchanged (5.9 vs 5.7). Our findings show integrated reporting has markedly increased the availability of aspirate reports to the histopathologist, and improved diagnostic concordance. This new model benefits the haematologist, histopathologist and patient.

The Value of Clonality in Lymphoma Diagnosis: Experience of a Tertiary Referral Molecular Laboratory

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Introduction: Clonality studies are carried out when the diagnosis of lymphoma is particularly challenging. The detection of clonality in lymphoproliferative lesions suspicious for lymphoma can be a valuable supplementary tool as it has a high positive predictive value. Clonal studies can also help to distinguish recurrent or residual disease from reactive inflammation. However false positive and negatives are common and can be attributable to several factors, including poor DNA quality.
Methods: 206 cases reported over a six month period (Jun-Nov 2014) were retrospectively reviewed, 62% of which were referral cases. We investigated various aspects of clonality studies; including DNA quality, fixation method, clinical information provided and correlation between the morphological/immunophenotypical findings and clonality results using Euroclonality/BIOMED-2 primers (IgH, IgK, TCR-B and gamma-delta).
Results: 166 (81%) had adequate DNA quality, 29 (14%) poor DNA and 11 (5%) had inadequate DNA quality. External cases had better DNA quality in the majority of cases. Clinical information was provided in 75% of local cases and 53% of external cases. In 56% of cases clonality results supported the initial histological report. 9 cases showed clonal expansion despite a benign process on histology. 21 suspected cases lymphoma (13 B-NHL and 8 T-NHL) showed no clonality, 8 of which yielded poor DNA quality. Skin cases although had good DNA quality, usually had low number of neoplastic cells resulting in poor PCR products.
Conclusions: DNA quality is very variable and clinical information is often not provided precluding adequate assessment of clonality findings. DNA was worse locally (decalcified marrow trephines using formic acid and Peloris system with high temperatures that can cause DNA degradation). Standardisation of fixation methods and interpretation of peaks/bands in the clinical context of the patient is essential for clonality to be informative.

Kikuchi-Fujimoto Disease: A Novel Diagnosis by Transbronchial Biopsy of Mediastinal Lymphadenopathy

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Objectives: Kikuchi-Fujimoto disease (KFD) is a rare, self-limiting form of necrotising lymphadenitis that most commonly affects young Asian women, and classically presents with fever, malaise and lymphadenopathy. The cervical lymph nodes are involved in around 85% of cases, with other sites rarely involved. We report an unusual case in which an unexpected diagnosis of KFD was made via transbronchial biopsy of mediastinal lymph nodes.
Methods: A 15 year old boy of Pakistani origin presented with a 4 month history of lethargy, neck stiffness and weight loss, with fever (up to 40°C) and night sweats. Chest X-ray, Mantoux test, blood cultures and viral PCR were negative. Lumbar puncture was normal, with no acid fast-bacilli. CT showed enlargement of the deep cervical lymph nodes (PET-positive on further imaging), and mediastinal lymphadenopathy. He was transferred to our hospital for further management, with a differential diagnosis of TB, lymphoma, rare infection or autoimmune disease. He underwent transbronchial biopsy of the mediastinal lymph nodes.
Results: His biopsy showed blood clot and cores of lymph node, with focal collections of crescentic macrophages, admixed lymphocytes and prominent apoptotic debris. Immunohistochemistry demonstrated a population of CD123-positive plasmacytoid dendritic cells and granular MPO positivity in macrophage cytoplasm. The background lymphocytes were mainly CD8-positive T-cells. The features were those of KFD.
Conclusion: Involvement of deep lymph nodes is unusual in KFD, and to our knowledge, this is the first case diagnosed via transbronchial biopsy. Such biopsies often produce scanty diagnostic material, and here the detection of the characteristic immunoprofile of KFD helped confirm the diagnosis. This case highlights that KFD should be considered at sites other than the cervical lymph nodes, and demonstrates the value of immunohistochemistry in reaching a definitive diagnosis.

CD30 Positive Intravascular T Cell Lymphoproliferative Disorder (ivTLPD) Presenting as a Pilonidal Abscess

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We describe a benign intravascular proliferation of atypical polytypic CD30 positive T cells, co-expressing follicular T helper cell lineage markers coincidental to local sepsis of the buttock. A 43 year old female presented with a 5x5cm buttock abscess at the site of a longstanding palpable lump. Peripheral blood showed only a neutrophilic leucocytosis. Immunohistological examination showed large aggregates of atypical CD30 positive, ALK negative lymphoid cells expressing a pan T helper phenotype with CD7 partially downregulated. Podoplanin proved that the atypical T cells were primarily, but not exclusively, localised within lymphatic channels. A T cell receptor clone was not detected using PCR. To find intravascular concentrations of atypical lymphoid cells is uncommon in skin biopsies and raises the possibility of leukaemia or intravascular lymphoma. Intravascular lymphoma is a rare variant of non Hodgkin Lymphoma with a minority possessing T or NK cell lineage but frequently involving skin. CD30 is a transmembrane glycoprotein and a member of the TNF superfamily involved in regulating proliferation. It is considered a reliable marker of lymphoma. Primary cutaneous CD30 positive TLPDs encompass a spectrum of biological aggressiveness and include primary cutaneous anaplastic large-cell lymphoma and lymphomatoid papulosis (LyP). CD30 can also be up regulated in activated B and T cells and it has been proposed that CD30 positive ivTLPDs are equivalent to an intravascular form of LyP. Intravascular proliferations of atypical CD30 positive T cells have been linked with chronic inflammation and abscess formation. Furthermore atypical CD30 positive TLPD expressing a CD4 positive T helper phenotype and exhibiting an indolent clinical course have been reported in the arm, trunk, neck and prepuce. Ultimately ivTLPD may require follow up based upon clinical features and natural progression due to overlapping diagnostic features.

Double Immuno-Staining in Lymphoma – A Pilot Study

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The investigation of lymphoid neoplasia requires multiple sections, in our practice consisting of twelve antibodies and thirteen single stained slides. By selecting particular antibodies for double staining, spatial relationships between cell types and overall tissue organisation can be more easily visualised. This pilot study aimed to optimise the staining intensity and specificity to provide accurate diagnostic information, reduce slide number, material and consumables costs, preparation time in the laboratory and storage space to enhance cost-benefit. Twelve antibodies were chosen and paired: kappa/lambda, CD3/CD20, MUM-1/CD10, Cyclin D1/CD30, BCL-6/CD5 and CD5/PAX-5. Firstly, these combinations were applied to normal tissue and then known tumours to optimise technique. Once the staining protocols were finalised they were run on eleven consecutive cases with conventionally stained Non-Hodgkin Lymphoma (NHL) panel requests. The slides were then reviewed by the Lymphoma team for quality and diagnostic accuracy compared to the standard single stained slides. The results demonstrate that double staining is possible in the diagnosis of NHL. The combinations chosen have proved successful and have provided interpretable results; for example, the relationship of light chain staining in plasma cells, MUM-1 and CD10 positive cells in Diffuse Large B-cell Lymphoma proves positive. We feel time will be saved cutting sections to improve efficiency in Lymphoma investigation and reduce panel storage space by around 30%. In conclusion the outcomes from this pilot study have been positive for medical and scientific staff, has shown that double staining in the diagnosis of NHL is possible and that optimising this protocol with a view to live diagnosis is worthwhile.

Using Image Analysis Software to Map in Vitro Response to Tyrosine Kinase Inhibitors in Chronic Lymphocytic Leukaemia

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In an Age in which Novel therapies are not necessarily defined by their ability to kill malignant cells, understanding the biology of malignant cells after treatment is extremely important. This is particularly true of Ibrutinib (Pcl-32765) therapy in chronic lymphocytic leukaemia which is characterised by lymphocytosis. Imagej/Fiji image analysis software could therefore be used to analyse cell shape and grouping characteristics. We used a novel assay in which Chronic Lymphocytic Leukaemia cells, cultured for 5 days, were seeded onto fibronectin coated glass coverslips and then had their B cell receptors ligated with goat anti human Igm. They were compared with cells simultaneously inhibited with Ibrutinib. We tested multiple staining techniques and found that using either Rose Bengal or Texas Red Phalloidin staining produced the most reproducibly analysable data when using Imagej/Fiji. We demonstrated, using the assumption that the outline of interacting cells would be larger than cells which were alone that Homotypic interactions were increased after B Cell receptor cross linking (54 groups larger than 2 cells against 35 group larger than 2 cells per 500nm X 250nm Field (P=0.019)). Nuclei were also significantly larger when cross linked. (Mean 33.23 (+-0.43) pixels without cross linking and 44.15(+-0.57) (P=≤0.0001)) suggesting increased nuclear spreading. These effects were reversed by the addition Of Ibrutinib with 18 groups larger than 2 cells per the same field(P=0.0252 compared with cross linked sells) and Nuclear size mean being 33.83 (P≤0.0001 compared with cross linked cells). This study demonstrates a novel, easily reproducible assay to assess a variety of cellular responses to Ibrutinib therapy and suggests a method of quantifying activity both when stimulated and inhibited. This technique could easily be scaled up to further investigate cellular behaviour following Ibrutinib therapy.

Lymphangiomatous Polyp of the Tonsil: An Unusual Mimic of Lymphoma

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Purpose of the Study: Lymphangiomatous polyp of the tonsil is a rare benign tumour, less than 30 cases of which have been reported. Such lesions typically present with symptoms of dysphagia, sore throat or throat mass. We report a case of lymphangiomatous polyp of the tonsil as a histological mimic of lymphoma.
Method: A 16 year old male with no significant past medical history presented with a symptomatic lesion of the right tonsil and underwent excision biopsy under general anaesthetic. The specimen was submitted for histological examination.
Results: Initial histological assessment of the excision specimen showed a polypoid lesion with surface squamous epithelium overlying loose connective tissue and dilated lymphatic channels. The lymphatic channels were engorged with dense aggregates of small, monomorphic lymphoid cells. The differential diagnosis included both lymphangiomatous polyp and lymphoma. Immunohistochemical staining confirmed the presence of D2-40 positive lymphatic spaces and a mixed population of CD4 positive and CD8 positive T cells, excluding lymphoma and confirming the diagnosis of lymphangiomatous polyp.
Conclusions: Lymphangiomatous polyp of the tonsil is a rare entity, which histologically may be confused with lymphoma. Immunohistochemical staining for D2-40 is helpful in confirming the diagnosis and a panel of lymphoid cell markers may be useful to exclude lymphoma.

Histopathological Examination of Asymmetrical Tonsils

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Purpose of the Study: It is now universally accepted across the UK that tonsillectomy specimens are not submitted for histological examination routinely, but only when there is some clinical indication to do so. Asymmetrical tonsillar enlargement may indicate malignancy or it can be due to many causes including repeated inflammation and chronic infection. Some researchers included additional clinical features that were common when there was malignancy in the asymmetrical tonsils. In this study we aim to evaluate the cancer detection rate in this setting including those who possess high risk clinical features such as age (> 45), pain and history of smoking.
Methodology: In total, 119 consecutive tonsillectomy cases, clinically labelled as asymmetrical tonsils were analysed. Out of these, 28 cases (Group 1) were additionally labelled with investigation for unknown primary, obvious lesion or suspicious ulcer seen. The remaining 91 cases with a sole clinical indication of asymmetry were stratified as group 2.
Results: In the first group, 7 cases had a histological confirmation of tonsillar primary (25%). While, in the second group, the histological analysis showed 78 cases with benign reactive pathology (89%) while, one case was diagnosed as malignant lymphoma (1%), 6 cases with mild dysplasia (7%), one case with moderate dysplasia (1%) and two cases were diagnosed as indefinite for dysplasia (2%).
Conclusion: Our results do correlate with the considerable agreement amongst otolaryngologists that the appearance of an asymmetrically enlarged tonsil in the presence of associated risk factors is considered an indication for tonsillectomy and histological examination given the significant rate of tonsillar malignancies in this group. Anatomical difference in the depth of tonsillar fossa and asymmetry of the anterior tonsillar pillar may give a false impression of a clinically asymmetrical or unilateral enlarged tonsil.

Metastatic Adenoid Cystic Carcinoma to the Lung and Kidney: A Single Case Report

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Adenoid cystic carcinoma (ACC) is a rare, slow growing and aggressive malignancy which arises within secretory glands, primarily the salivary glands, with a predilection for perineural spread and propensity for late, local recurrence and distant metastasis. The tumour has three prognostically significant subtypes which form the basis for tumour grading; cribriform, most frequent, tubular and solid, associated with a more aggressive clinical course and metastasis. In the majority of cases (74%) the tumour has an insidious onset and patients have locally invasive disease at first presentation, which coupled with adjacent, important anatomical structures means a complete primary surgical resection is often not feasible. Distant metastases are frequent and predominately involve the lung and bone with renal metastasis a rare occurrence. An optimal treatment regime for ACC has yet to be established and while local management with combination surgery and adjuvant radiotherapy is currently favoured there is conflicting evidence regarding the use of radiotherapy and no formal surveillance guidance for local and regional recurrence or distant metastasis exists. We report a single case of a 52 year old male who presented in 2004 with an asymptomatic right neck mass and underwent right radical neck dissection and adjuvant radiotherapy for ACC. The patient represented in 2014 with aspiration pneumonia on a seven month history of dysphagia and dyspnea. Xray and subsequent computed tomography (CT) imaging identified large, multiple right sided lung lesions, maximum 3cm, and a 5.6cm left renal upper pole mass. CT guided biopsy of the right lung lesion and ultrasound guided biopsy of the renal mass were reported as metastatic adenoid cystic carcinoma, c-KIT negative. The patient underwent a left nephrectomy in February 2015 and is awaiting further review regarding his lung metastasis.

Cytokeratin 7: A Novel Biomarker in Human Papillomavirus-Related Oropharyngeal Squamous Cell Carcinoma

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Purpose: Cytokeratin 7 (CK7) is a junctional biomarker with a SEQIKA fragment which stabilises HPV-16 E7 transcripts. We assessed the expression pattern of CK7 protein in tumour specimens from patients diagnosed with oropharyngeal squamous cell carcinoma (SCC) presenting at two major Irish head and neck centres, within the last 10 years.
Methods: Archived tumour specimens together with epidemiological data were collected from patients presenting with new primary oropharyngeal SCC at two main head and neck centres in Ireland, within the last 10 years. Briefly, DNA was extracted from tissue blocks and HPV testing carried out using SPF10 HPV PCR. HPV positive cases were evaluated using the INNO-LiPAHPV Genotyping Extratest [Fujirebio]. Immunohistochemical staining for CK7 [Clone SP52, Ventana] was performed on tissue blocks following optimisation on the Ventana BenchMark Ultra Immunostainer. Slides were analysed by light microscopy and scored using the H scoring system. CK7 expression was correlated with epidemiological data, p16ink4a positivity and HPV status. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS).
Results: There were 220 cases of oropharyngeal SCC. 41% demonstrated p16ink4a positivity and 44% demonstrated HPV positivity, with 95% of these identified as HPV-16 subtype. CK7 expression was observed in the tonsillar crypt epithelium of both normal tonsils and tumour specimens. 56% of cases were positive for CK7, with 38% of cases demonstrating H score of >60. CK7 expression in the tumour cells was significantly linked to HPV status and p16ink4a-positivity ($p<0.05$).
Conclusions: We present for the first time expression of CK7 in the tonsillar crypts of oropharyngeal cancers. Our results suggest that the expression of CK7 in normal tonsillar crypt epithelial cells provides a selective advantage to HPV-related carcinogenesis at this site, possible due to the unique propensity of CK7 to bind and stabilise HPV-16 E7 transcripts.

Junctional Biomarkers and Immune Evasion in Human Papillomavirus-related Oropharyngeal Squamous Cell Carcinoma

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Purpose: HPV-related oropharyngeal SCC represents a distinct clinicopathological subgroup of head and neck tumours. Pathogenesis of this disease in an immune-rich site is poorly understood. There are also clear similarities to SCC of the cervix. We analysed the expression of biomarkers of immune evasion and translation of cervical junctional biomarkers.
Methods: Archived HPV-positive tumour specimens and epidemiological data were collected from patients presenting with new primary oropharyngeal SCC at two head and neck centres in Ireland over a one year period. Briefly, DNA was extracted from tissue blocks and HPV testing carried out using SPF10 HPV PCR. The INNO-LiPA HPV Genotyping Extra test [Fujirebio] was used to determine genotype. Immunohistochemical staining for CK7, GDA, MMP-7, AGR-2, PD-1 and PD-L1 was performed following optimisation. Slides were analysed by light microscopy and scored using the H scoring system (junctional biomarkers). Expression was correlated with tumour, clinical and epidemiological data. Statistical analysis was performed using SPSS.
Results: Sixteen specimens of HPV-related oropharyngeal SCC were included and five specimens of HPV-negative oropharyngeal were also stained. 15/16 demonstrated p16ink4a positivity. HPV subtypes 16 (16 cases), 33 (1 case) and 44 (1 case) were identified. Junctional biomarkers were expressed in tonsil crypt epithelium and to varying degrees in tumour specimens. Expression of PD-1 (13 cases) and its ligand (14 cases) were interpreted qualitatively, based on expression pattern, often presenting at the periphery of tumour islands.
Conclusions: We have identified markers that selectively identify tonsillar crypt cells associated with HPV oncogenic infection and which correlate with cells in the cervix where top-down differentiation in SCC occurs. Markers of the PD-1:PD-L1 immune checkpoint pathway are identified and suggest a role for this immune complex formation in immune evasion in this subgroup of SCC.

What is the Extent of Inter-Tumour Heterogeneity in Patients with Pancreatic Neuroendocrine Tumours?

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Introduction: Pancreatic neuroendocrine tumours (pNETs) are classified under ENETS grading system. This system uses Ki-67 as a prognostic marker. Paraffin-embedded tissue from either primary or secondary tumours may be used for the production of Ki-67 labelling index (LI). Intra-tumour heterogeneity has been observed. It is not fully understood to what extent inter-tumour heterogeneity occurs and how tumour selection, for Ki-67 staining, may affect reported patient tumour grade.
Aims: To determine the extent of inter-tumour, intra-patient heterogeneity within pNETs. To determine whether tumour selection can alter patients' tumour grade and thus predicted prognoses.
Material and Method: Patients were selected from NET database, consisting of 513 patients. 17 patients were included within this study. Patients were included if they had a confirmed pNET, multiple resected specimens, and the presence of research consent. Ki-67 staining was performed on all resected specimens meeting the inclusion criteria. 2000 cells were counted in areas of Ki-67 hotspots to produce a Ki-67 LI. Comparison was made between primary and secondary Ki-67 LIs.
Results: We have looked at 17 patients, taking into account over 75 specimens. Preliminary results suggest that inter-tumour variability exists between primary and separate secondary pNETs. Additional data will be provided.
Keywords: Ki-67, Pancreatic, Neuroendocrine, Tumour, and Inter-Tumour Heterogeneity.

Frozen Section Reporting of Necrotising Granuloma of the Liver Following Percutaneous Instrumentation of The Biliary Tree: A Case Series

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Percutaneous transhepatic cholangiography (PTC) is an interventional radiological technique for both diagnostic imaging and therapeutic decompression of the proximal biliary tract in malignant distal obstruction when retrograde techniques fail. Recognised complications of PTC include sepsis, haemorrhage and pneumothorax. We describe four cases where necrotising granuloma, apparently secondary to previous PTC, has resulted in frozen section examination at the time of subsequent planned cancer resection, to exclude tumour metastasis. Four cases of necrotising granuloma in the liver have been identified between January 2013 and February 2015. All cases were planned Whipple's procedures for pancreatic cancer where initial intraoperative evaluation revealed solitary subcapsular liver lesions. Biopsy and intraoperative frozen section examination were performed to exclude metastatic disease. All frozen sections except one were reported as showing benign necrotizing granuloma formation. The first case was initially reported as malignant and the operation was abandoned. A benign diagnosis was confirmed on paraffin sections in all four cases with the first patient undergoing successful surgical resection at a later date. To the best of our knowledge these are the first reported cases of necrotising granuloma in the liver secondary to prior instrumentation of the liver and leading to intraoperative histological assessment. We highlight this as a potential pitfall in frozen section interpretation undertaken ahead of planned potentially curative surgery which can lead to overstaging of otherwise resectable disease or to the interpretation of a potential diagnosis of tuberculosis. These risks can be reduced with greater surgical and pathological awareness of this entity.

Inclusion Body Fibromatosis: A Case Report

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Inclusion body fibromatosis, also known as infantile digital fibroma, is a benign, predominantly myofibroblastic tumour primarily found on the digits of infants. Clinically, these lesions present as asymptomatic cutaneous nodules, rarely larger than 2cm in size, classically on the dorsal or dorsolateral aspect of the second, third and forth digits. They have a high recurrence rate, reported as between 61 and 75%, although this can be reduced by undertaking complete wide local excision. We report a case of inclusion body fibromatosis in an 11 month-old boy, presenting with an enlarging, firm lesion on his left second toe. Following surgical excision, the lesion showed classical histological features of inclusion body fibromatosis - spindle cells arranged in interlacing fascicles in collagenous stroma and numerous pink intracytoplasmic inclusions. The lesion appeared incompletely excised and the patient will be kept under review on account of the high risk of recurrence.

Decreased Expression of the Mitochondrial BCAT Protein Correlates with Improved Patient Survival in IDH Wild-Type Gliomas

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Purpose: Gliomas represent 43% of all solid intracranial tumours and are associated with a poor prognosis. Recent studies indicated that the human cytosolic branched chain aminotransferase protein (hBCATc), which metabolises the branched chain amino acids (BCAA), was significantly upregulated in IDH1/2 wild type (WT) glioblastomas, correlated with methylation patterns in the BCAT1 promoter and is associated with a worse prognosis compared with IDH mutant gliomas. The diagnostic and prognostic significance of markers of BCAA metabolism is currently under investigation.
Methods: 64 glioma tumour samples were compared for hBCATc, hBCATm and BCKDC expression using western blotting and immunohistochemistry. DNA was extracted from fresh frozen tissue. Sanger sequencing of the p.Arg132 region of IDH1 and p.Arg172 region of IDH2 was undertaken using a 3730 DNA analyser (Applied Bio-Systems). SUMMARY: In IDH WT tumours, like hBCATc ($p=0.007$), the expression of the mitochondrial isoform (hBCATm) is significantly ($p=0.036$) expressed relative to IDH mutant gliomas. hBCATm additionally shows a more significant correlation with patient survival than hBCATc on Kaplan-Meier analysis. In IDH WT tumours, low hBCATm expression is a positive prognostic factor ($p = 0.003$). hBCATm expression additionally correlated with WHO grade. Although previous reports indicate that increased hBCATc occurs exclusively in IDH-WT tumours, our studies demonstrate that 30% of IDH mutant tumours express comparable levels of hBCATc. Although hBCATc alone has been suggested as a putative therapeutic target, it is important to evaluate the expression of hBCATm in glioblastomas as its expression may impact the efficacy of new treatments targeting hBCATc.
Conclusions: IDH WT high grade gliomas traditionally have a poor prognosis. However we demonstrate for the first time that relatively low hBCATm may select for a better performing clinical cohort and may be a possible candidate target for drug therapy.

Myxoinflammatory Fibroblastic Sarcoma – A Rarely Described FNA Diagnosis with Histological Confirmation

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A 38 year old female presented with an occipital mass, presumed to be a lymph node and underwent fine needle aspiration of the lesion. FNA yielded two air dried slides, upon which a diagnosis of mesenchymal neoplasm was made. The patient underwent a subsequent incisional biopsy allowing a formal histological diagnosis of myxoinflammatory fibroblastic sarcoma to be made. Myxoinflammatory fibroblastic sarcoma is a low-grade neoplasm usually occuring on the distal extremities and only rarely presents as a head and neck neoplasm. FNA is a useful tool in the diagnosis and subsequent management of head and neck neoplasia and we describe here the cytological features and subsequent histological diagnosis of myxoinflammatory fibroblastic sarcoma occuring in the occipital scalp.

Giant Cell Fibroblastoma: A Case Report

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Objective: Presentation of giant cell fibroblastoma (GCF). Because of it has a dilemma of microscopic appearances; it is mandatory to differentiating it from atypical dermatofibroma (ADF), fibrous hamartoma of infancy (FHI) and vascular lesions.
Methods: Eighteen month-male Egyptian child presented with painless slowly expanding subcutaneous back swelling at the left scapular area.
Results: Histologically, the lesion is poorly circumscribed and range from cellular to myxoid in a dense to loose collagenous stroma. The tumours composed of mixture of spindle shaped or stellate cells admixed with multinucleated giant cells with occasional pleomorphism and very low mitotic index (<1 per 50 high-power fields). These cells infiltrate around adnexal structures and through subcutaneous fat. A distinctive finding is cracking artifact of the stroma simulating angiectoid spaces. These pseudovascular spaces are lacking a true endothelial lining and lined by discontinuous layer of enlarged multinucleated giant cells. Immunostains, including factor VIII, CD31, CD1a, SMA, S100 and CD68 were negative. Ki 67 labeling index is very low. All the cellular components show positive immunoreactivity for CD34.
Conclusions: First case of GCF reported in Egypt. We recommend a wide scaled study to categorize this tumour with molecularly similar lesions.

An Unusual Case of a Splenic Leiomyoma

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A 60 year old man being investigated for obstructive hydronephrosis was incidentally found to have a 3.2cm splenic mass on computed tomography (CT). No lymphadenopathy was present and the mass remained stable on sequential CT and ultrasound scans. On positron emission tomography (PET) the lesion had a low signal with moderate uptake. All haematological investigations were within normal limits including a negative Epstein Barr Virus (EBV) test. His past medical history included previous immunosuppressive therapy for inflammatory bowel disease. A core biopsy under CT guidance was performed. The cores showed a paucicellular spindle cell lesion with bland, blunt ended nuclei, no cytological atypia and a sparse chronic inflammatory infiltrate. There was no necrosis. The spindle cells stained positive for smooth muscle actin (SMA) and H-Caldesmon indicating this to be a splenic leiomyoma. Splenic lesions are uncommon and within their differential include, lymphoma, inflammatory pseudotumour, harmatomas and leiomyomas.[(1)]A splenic leiomyoma is an unusual and rare benign smooth muscle tumour with an unknown pathogenesis. They are thought to arise from the capsule and blood vessel walls of organs.[(2)]They have been documented in immunosuppressed states (constitutional or acquired), in those with EBV infection and in children with ataxia-telangiectasia.[(2)]Leiomyomas within the spleen are rarely reported in the literature, especially in those patients over the age of eighteen. In this case there was historical immunosuppression, however leiomyomas should be considered in the differential diagnosis of well- defined solitary splenic lesions.
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The Impact of Roux-en-Y Gastric Bypass on Biochemical and Morphological Correlates of Glomerular Injury in an Animal Model of Type 2 Diabetes

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Purpose of the Study: The Zucker Diabetic Fatty (ZDF) rat is extensively used as a model of Diabetic Kidney Disease (DKD) associated with obesity and progressive insulin resistance ('diabesity'). This study aimed to validate qualitative ultrastructural parameters of glomerular injury in the ZDF animal model and apply these criteria to an interventional study investigating the effects of Roux-en-Y gastric bypass (RYGB) on DKD.
Methods: Superficial renal cortices were immersion-fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, processed and embedded in epoxy resin prior viewing under a Technai 12 transmission electron microscope. Glomerular basement membrane (GBM) thickness, podocyte foot process diameter (PFPD) and podocyte foot process frequency (PFPF) per unit length of GBM were determined for each group (Sham and RYGB operated ZDF fa/fa diabetic animals vs non-operated non-diabetic ZDF fa/+ lean controls). Statistical analysis was performed using a Mann Whitney U test and an unpaired t-test where appropriate.
Summary of Results: Selected TEM parameters (GBM thickness, PFPD and PFPF) demonstrated significant differences between specified Sham-operated ZDF fa/fa vs fa/+ samples, p=0.017. *Analysis of RYGB interventional study samples still in progress*. Early post-operative glucose measurements showed a significant improvement in glucose homeostasis in the RYGB group (RYGB vs SHAM, P=0.0001) occurring independently of weight loss. Urinary albumin:creatinine ratios were lower in the RYGB group vs Sham operated positive controls (P=0.0079) and were comparable with age-matched lean control fa/+ samples.
Conclusions: Preliminary findings support a beneficial role for RYGB in an animal model of 'Diabesity'. Validated ultrastructural parameters should assist in elucidating changes in podocyte activation and differentiation as mediators of the observed remission of albuminuria following RYGB surgery.

P16, Ki67 and HMB45 Expression in Spitz Nevi: Comparison with Melanomas and Common Nevi

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Spitz naevus is a benign melanocytic lesion that shares many histological features with malignant melanoma. Although the morphological criteria differentiating the two entities are well established however, some cases can be challenging. Many isolated markers have been proposed to help in differentiating Spitz naevus from melanoma, albeit none has been shown to be definitive.
Aim: This is a preliminary study looking at the immunohistochemical expression of 3 markers that are known to have important role in cell cycle regulation, proliferation and melanocytic differentiation (P16, Ki67, and HMB45). The aim is provide to a combination of proteins that can help in differentiating Spitz naevus from malignant melanoma.
Methods: The study included 12 cases of Spitz naevi, 6 benign compound naevi and 6 cases of malignant melanoma. Immunohistochemical expression of p16, Ki67, and HMB45 has been accessed and compared with the morphological features of these lesions.
Results: It is noted the mean P16 expression is higher in compound and spitz naevi than melanoma (83, 91, and 36 respectively). Proliferation activity as measured by KI67 index is higher in melanoma in comparison with compound and spitz naevi (30.8, 2.8, and 1.6 respectively). HMB45 shows only junctional positivity in 9 out of 11 cases of Spitz naevi while in the other two it shows week dermal component. HMB45 is constantly positive at the deep dermal component of melanoma, albeit the staining intensity is variable.
Conclusion: The immunoprofile of Spitz naevus is different from that of a malignant melanoma. A combination of biological markers as (p16, Ki67, and HMB45), can provide a potential tool to differentiate between the two entities. Nevertheless, expanding the biomarker repertoire on a large number of cases is necessary to further establish a reliable panel to differentiate among difficult cases.

Direct Immunofluorescence in a Tertiary Referral Centre: An Audit of Local Guidelines and Usage

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Direct immunofluorescence (DIF) forms an important and costly adjunct to conventional haematoxylin and eosin (H&E) histology in dermatopathology, particularly in bullous diseases and other immune-mediated diseases. We aim to assess the usage and diagnostic yield of DIF in our dermatopathology department. 134 requests for DIF on skin biopsies received over a 5 month period met the inclusion criteria. Each individual report was assessed with regard to the indication for DIF, whether DIF was deemed to be indicated or not indicated on assessment of the clinical history supplied on the request card, the results of DIF and whether DIF was contributory to the final diagnosis. We also collected data on the usage of DIF over the last 2 years to assess changes in practice. All 134 requests for DIF were granted in line with current departmental policy. The indication categories were divided as follows: bullous 41, alopecia 4, lupus 32, vasculitis 23, dermatitis herpetiformis (DH) 15 and 'other' 19. All requests for DIF were deemed to be indicated in both the bullous and DH categories by our panel, but indicated requests varied from 8.7% to 50% in the remaining categories. In 45.5% (61 out of 134) of cases DIF was deemed to be contributory to the final diagnosis. Our analysis also showed that usage of DIF in our department is escalating, with a 23.2% increase in requests from 2013 to 2014. Our departmental policy with regard to DIF is inclusive and operates solely on the basis of clinician request. With the increasing usage of DIF, established departmental guidelines and/or a protocol for DIF usage should be mutually agreed with dermatology colleagues in order to ensure effective use of this expensive test.

Overview of Merkel Cell Carcinoma in an Irish Population

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Purpose of Study: Merkel cell carcinoma (MCC) is an uncommon but highly aggressive primary cutaneous malignancy of neuroendocrine cells with a propensity for regional and distal metastases. Due to it's rarity, information relating to it's epidemiology in an Irish population is limited, mainly owing to difficulty in gathering large patient series. Our aim was to identify all cases of MCC in our institution in a defined 10 year period and review the patient demographics compared to internationally available data.
Methods: A search was carried out on the hospital laboratory system to identify all cases of MCC from 01/01/2005 to 21/12/2014. All histology reports were reviewed and any information pertaining to patient demographics was recorded in an excel spreadsheet. A literature review was performed relating to the patient profile of MCC internationally and the results were compared.
Results: A total of 33 reports pertaining to 25 individual patients were recovered. All patients were of Caucasian Irish ethnicity. The incidence of MCC was higher in men (56% of cases, n=14) than women. The median age at diagnosis was 80 years (range 57-90). Men presented at an earlier age (median 78 years) than women (median 83 years). Regarding the anatomic site of the tumours, 64% (n=16) were on the head or face, 20% (n=5) were on the lower limb and 12% (n=3) were on the upper limb. All were on sun-exposed sites. Of note, the majority of tumours in the male population were on the head (78.6%, n=11), while the female population showed an equal distribution between the head and the lower limbs (45%, n=5 for each sub-site).
Conclusions: The subset of patients we identified show demographics consistent with published literature for US, Australian and other European cohorts. Merkel Cell Carcinoma is a disease of the elderly affecting sun-exposed sites. We note some variation in the dominant anatomic sites between genders and conclude this is due to differing environmental exposure.

A Platform for Single Cell Manipulation and Analysis (PASCA)

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Background: A novel cell-dispensing instrument referred to as a Single Cell Manipulator (SCM) device was developed with the following features: i) rapid optical and fluorescent detection of single cells ii) generation of picoliter sized droplets encapsulating the isolated single cell and iii) printing of the single cell in an "ink-jet" like manner onto a chosen substrate. This technology was used to isolate cells of interest from i) heterogeneous mixed populations of cells, ii) co-cultures of cells and iii) clinical patient samples for subsequent downstream biological analysis.
Methods: Cells were injected into a reusable silicon dispenser chip that was coupled to a live cell camera for image capture and display of cells approaching the chip's exit nozzle. An optical detection mechanism determined the presence of single, fluorescent cells within the selected region of interest close to the chip exit nozzle. A sorting algorithm ensured that only droplets containing the single cells of interest were selected for printing to the prescribed location and user-chosen substrate.
Results: Fluorescently labelled HPV16 CaSki cervical cells were spiked into a cervical liquid based cytology sample and printed onto a glass slide using the SCM. Undifferentiated NTera2 human embryonal cancer stem cells were isolated from a mixture of differentiated and undifferentiated cells based on fluorescent tagging of the cell surface receptor, stage-specific embryonal antigen 4 (SSEA4). The thyroid stimulating hormone receptor (TSHR) was expressed in anaplastic V600E mutated thyroid cancer cell lines that were treated with the MEK inhibitor PD0325901. Treated cells were isolated using the SCM.
Conclusion: The SCM PASCA technology allows isolation of single cells from heterogeneous populations of cells and clinical samples for downstream analysis at a single cell level.

Protein Quantification in IHC Stained Human Cell Lines Using Manual Image Analysis for Calibrated Analysis of Tissue Microarrays

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This study aims at quantifying immunohistochemistry (IHC) stained human cell lines for protein biomarkers by manual pathologist review. Staining analyses are used to calibrate tissue microarrays of tumour cores, against quantitative protein concentration allowing a systems-based data analysis. As a proof of concept, FFPE human cell line pellets (n=13) were IHC stained for Smac protein and analysed using Aperio image analysis software. Staining quantification manually performed by pathologists provided parameters including average staining intensity, percent total cell positivity and H-score. These data were enriched by qualitative parameters pointing out possible histological artefacts. A calibration curve was plotted using H-score data and protein concentrations, previously determined by Western blotting. The panel of cell lines provided a range of strong and weak/absent IHC staining using a highly specific Smac antibody. The calibration curve showed a strong correlation between absolute protein concentrations and manual H-scores. Expression amounts in cell lines correlate with IHC staining intensities determined by pathologist review. The linear correlation between manual H-score and absolute protein values provides an avenue to indirectly determine protein expression. Further analysis will be performed on additional antigens and analysis outcomes will be then compared to digital results. This data will provide the basis for deterministic systems-biological data analysis approaches.

'Lean' Laboratory Requests: A Mobile App for Molecular Test Requests

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Purpose of the Study: 'Lean' is a management framework for maximising value and minimising waste. It originated in the automotive manufacturing industry and has been utilised successfully in non-manufacturing processes. One such application in our department was the 'leaning' of the molecular test requesting process using a smart-phone app. This study will look at the potential utility of this application within the National Health Service (NHS), wherein approximately twenty different molecular test request forms are currently in use.
Methods: A mobile application to facilitate molecular test requesting was developed using Xcode and the objective C programming language. The application was built around an email based system. Patient anonymity was paramount in the design; NHS numbers are used as identifiers. The application generates a molecular test request form and can also generate a national cancer drugs fund application form for each request. Administrative staff use colour coded flags to represent the progress of each email request through the workflow process to facilitate tracking.
Summary of Results: The app reduced the administrative staff workload by reducing the number of steps and paperwork involved in the molecular test requesting process. A three-fold reduction in time taken by clinicians to request molecular tests was noted. A survey of staff involved with molecular test requesting revealed a 90% reduction in 'lost requests' after the introduction of the application.
Conclusions: We present a 'lean' method for requesting molecular tests using a smart-phone app. This application can be used to standardise molecular test request forms within the NHS along with automatic generation of a national cancer drugs fund application form for each request.

How Do Variations In Whole Slide Scanner Hardware Components and Scanning Processes Affect Digital Image Analysis Results from Scanned Microscope Sections?

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Purpose of Study: Automated approaches for quantitative digital image analysis (DIA) of tissues are becoming increasingly popular in pathology due to advances in whole slide scanning hardware and digital imaging technology. It is essential that DIA is standardised to ensure accuracy and reproducibility of results. Very limited published data exists on the effect of scanner hardware variations on the accuracy and reproducibility of DIA results.The aim of this study was to test the following variables: variation in light source intensity during the day; presnap calibration & white balance of scanned images; variation in DIA due to debris on peripheral parts of the section or coverslip edges.
Methods: Immunohistochemistry stained sections from 3 patient samples were scanned on the same Aperio CS scanner, hourly, for 2 consecutive days to generate 15 scanned images of each sample, representing 45 images in total. All scanned images were run through Aperio software using a macro to quantify positive cell counts. For a subset of images, a region of interest was drawn around the tissue to exclude any debris/coverslip edges from peripheral parts of the slide in the subsequent DIA. Statistical analysis was performed to calculate the coefficient of variation between DIA results from scans on different days.
Results: Variation in light source intensity accounted for 1.2% to 2.1% variation in cell counts between repeat scans of the same slide. Exclusion of debris/coverslip edges accounted for 1-2% variation in cell counts between repeat scans of the same slide. Subjective analysis revealed no significant difference in appearance of different scans of the same slide.
Conclusion: Variation in light source intensity, presnap calibration and overall white balance, plus debris in peripheral areas of the section account for minimal variation in resultant DIA results. Technical advances in scanner hardware have reduced variability in scanning operations. Further investigations are ongoing.