Summer Meeting Programme 2010

29 June – 1 July

❁

198th Scientific Meeting of the Pathological Society of Great Britain & Ireland

❁

Co-hosted by:
The Bute Medical School, University of St Andrews
The Centre for Oncology and Molecular Pathology, University of Dundee

❁

Companion Sessions:
Association of Clinical Electron Microscopists
Renal EQA

❁

Venue:
School of Physics and Astronomy,
University of St Andrews, North Haugh,
St Andrews, Scotland KY16 9SS

PROGRAMME ACKNOWLEDGEMENTS

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TUESDAY 29 JUNE 2010

FOYER
09.00 **Registration and Coffee**

ROOM 235 *(BETWEEN LECTURE THEATRES Ⓐ AND Ⓑ) – Microscopes*
COMPUTER ROOM (GROUND FLOOR) *– Virtual slides*
10.00–17.30 **Slide Seminar Case Viewing**: ***Head / Neck / Oral Pathology***

LECTURE THEATRE Ⓐ
10.00–10.15 **Opening Ceremony**
Chair: Prof CS Herrington, University of St Andrews and Prof S Fleming, University of Dundee
Speaker: Dr L Richardson, Principal, University of St Andrews

10.15–13.00 **Symposium: *Cell Signalling***
11.15–11.45 Coffee **[FOYER]**

FOYER	**LECTURE THEATRE Ⓒ**
13.00–14.30 **Lunch and Trade Exhibition**	**13.30–14.30** **Trainees Session – Meet the Experts: *Renal Tumour Pathology***

FOYER
14.30–15.30 **Poster Viewing / Rounds** (including tea/coffee)

LECTURE THEATRE Ⓐ	**LECTURE THEATRE Ⓑ**
15.30–17.30 **Oral Presentations**	**15.30–17.30** **Oral Presentations**

LECTURE THEATRE Ⓐ
17.30–18.30 **Public Lecture**
Speaker: Prof J Ironside, CBE, University of Edinburgh, National CJD Surveillance Unit

PARLIAMENT HALL (University of St Andrews)
18.30–20.00 **Welcome Reception**

All details are subject to amendment
Visit our website for further information and updates: **www.pathsoc.org**

Programme Quick Reference Tables

WEDNESDAY 30 JUNE 2010

FOYER

08.00 **Registration and Coffee**

ROOM 235 *(BETWEEN LECTURE THEATRES* **A** *AND* **B***) – Microscopes*

COMPUTER ROOM (GROUND FLOOR) *– Virtual slides*

09.00–17.30 **Slide Seminar Case Viewing**: ***Head / Neck / Oral Pathology***

LECTURE THEATRE A		**LECTURE THEATRE C**	
09.00–12.00	**Oral Presentations**	**09.00–12.00**	**Trainees' Symposium:** ***Molecular Pathology:*** *What it is and what can it tell us?*
10.30–11.00	Coffee **[FOYER]**	10.40–11.10	Coffee **[FOYER]**

LECTURE THEATRE A

12.00–13.00 **Keynote Lecture:** ***Colorectal Cancer Screening – Pilot to Progamme***
Speaker: Prof R Steele, University of Dundee

FOYER

13.00–14.00 **Lunch and Trade Exhibition**

FOYER		**LECTURE THEATRE C**	
14.00–15.00	**Poster Viewing and Chairman's Rounds**	**14.00–15.00**	**Renal Pathology EQA**

LECTURE THEATRE A		**LECTURE THEATRE B**	
15.00–17.30	**Oral Presentations**	**15.00–17.00**	**Symposium:** ***Pathology in the Developing World***
16.00–16.30	Tea **[FOYER]**	16.00–16.30	Tea **[FOYER]**

All details are subject to amendment
Visit our website for further information and updates: **www.pathsoc.org**

Programme Quick Reference Tables

THURSDAY 1 JULY 2010

FOYER

07.30 **Registration and Coffee**

LECTURE THEATRE C

07.45–09.00 **Trainees' Breakfast Session: *Ovarian Cancer Pathology***
(Breakfast will be served)

ROOM 235 *(BETWEEN LECTURE THEATRES A AND B) – Microscopes*
COMPUTER ROOM (GROUND FLOOR) *– Virtual slides*

09.00–17.30 **Slide Seminar Case Viewing**: ***Head / Neck / Oral Pathology***

LECTURE THEATRE A		LECTURE THEATRE B		LECTURE THEATRE C	
09.00–13.00	**Symposium: *Viruses and Disease***	**09.00–13.00**	**Oral Presentations**	**09.20–17.00**	**Association of Clinical Electron Microscopy**
11.00–11.30	**Coffee**	**11.00–11.30**	**Coffee**	**10.30–11.00**	**Coffee**

FOYER

13.00–14.00 **Lunch and Trade Exhibition**

LECTURE THEATRE B

13.30–14.30 **Pathological Society Annual Business Meeting**

FOYER

14.00–15.00 **Poster Viewing and Chairman's Rounds** *(tea/coffee will be served)*

LECTURE THEATRE B

15.00–16.00 **Slide Seminar Case Review: *Head / Neck / Oral Pathology***

LECTURE THEATRE A

16.00–17.30 **Plenary Oral Presentations**

17.30–18.30 **Pathological Society's 7th Doniach Lecture**
Speaker: Prof CJL Meijer, Vrije University Medical Centre, Amsterdam

LOWER COLLEGE HALL

19.30–23.00 **Society Dinner**

All details are subject to amendment
Visit our website for further information and updates: **www.pathsoc.org**

COMPANION MEETINGS [LECTURE THEATRE C]

Wednesday 30 June	14.00–15.00	Renal Pathology EQA
Thursday 1 July	09.20–17.00	Association of Clinical Electron Microscopists

KEYNOTE AND NAMED LECTURES [LECTURE THEATRE A]

Tuesday 29 June	17.30–18.30	Public Lecture: *Brain Tissue Donation - a Unique Gift Essential for Neuroscience Research?* Prof J Ironside, CBE, Edinburgh
Wednesday 30 June	12.00–13.00	Keynote Lecture: *Colorectal Cancer Screening – Pilot to Programme* Prof R Steele, Dundee
Thursday 1 July	17.30–18.30	Doniach Lecture: *Back to the Future* Prof CJL Meijer, Amsterdam

ORAL COMMUNICATIONS

Tuesday 29 June	15.30–17.30	[LECTURE THEATRE A] and [LECTURE THEATRE B]
Wednesday 30 June	09.00–12.00	[LECTURE THEATRE A]
	15.00–17.30	[LECTURE THEATRE A]
Thursday 1 July	09.00–13.00	[LECTURE THEATRE B]

NOTE TO PRESENTERS
Speakers are reminded that no communication may exceed the time allocated on the programme without the consent of the meeting, obtained through the Chairman.

PLENARY ORAL SESSION [LECTURE THEATRE A]

The six highest-ranked submitted oral abstracts will be presented on Thursday 1 July, 16.00–17.30.

PRIZE
A prize for the best presentation, donated by the *Journal of Pathology* will be presented at the Society Dinner.

POSTERS, VIEWING AND CHAIRMAN'S ROUNDS [FOYER]

POSTER SIZE
Poster boards will be size 1m x 1m. Please ***do not*** exceed these dimensions. Velcro will be provided.

VIEWING AND ROUNDS

Tuesday 29 June	14.30–15.30
Wednesday 30 June	14.00–15.00
Thursday 1 July	14.00–15.00

PRIZES
Poster round chairs will be circulating to select the winners of the Pathological Society's Sir Alastair Currie Prize and second and third poster prizes. Winners will be announced at the Society Dinner on 1 July.

NOTE TO PRESENTERS
Posters should be in place by 09.00 hrs on the day of presentation and **must be removed by 17.00 on the same day**.

PRESENTATION
The presenting author (or another contributor) must attend the meeting and present the poster during the allocated poster rounds in order for the abstract to be published in the *Journal of Pathology* on-line supplement after the meeting.

SLIDE SEMINAR COMPETITION AND REVIEW SESSION

Head / Neck / Oral Pathology

VIEWING

Virtual slides	**[COMPUTER ROOM – GROUND FLOOR]**
Microscopes	**[Room 235 –** *between lecture theatres* **Ⓐ** *and* **Ⓑ]**

COMPETITION
There will be a slide competition using slide images, which will be available during the following dates/times:

Tuesday 29 June	10.00–17.30
Wednesday 30 June	09.00–17.30
Thursday 1 July	09.00–17.30 *(competition closes at 15.00)*

PRIZE
A case of champagne. The winner will be announced at the Society Dinner on Thursday 1 July.
At the discretion of the winner, by tradition, this is shared amongst those present at the dinner!

DISCUSSION SESSION

Thursday 1 July	15.00–16.00	**[LECTURE THEATRE Ⓑ]**

SYMPOSIA

Tuesday 29 June	10.15–13.00	*Cell Signalling* **[LECTURE THEATRE Ⓐ]**
Wednesday 30 June	15.00–17.00	*Pathology in the Developing World* **[LECTURE THEATRE Ⓑ]**
Thursday 1 July	09.00–13.00	*Viruses and Disease* **[LECTURE THEATRE Ⓐ]**

TRAINEES' PROGRAMME [LECTURE THEATRE Ⓒ]

Tuesday 29 June	13.30–14.30	Meet the Experts: *Renal Tumour Pathology*
Wednesday 30 June	09.00–12.00	Symposium: *Molecular Pathology: What it is and what can it tell us?*
Thursday 1 July	07.45–09.00	Breakfast meeting: *Ovarian Cancer Pathology*

CONTINUING PROFESSIONAL DEVELOPMENT [CPD]

This Meeting has been approved by the Royal College of Pathologists for the purpose of Continuing Professional Development. Credits can be accrued as follows:

	FULL DAY	HALF DAY
Tuesday 29 June	7 credits	3 credits
Wednesday 30 June	7 credits	3 credits
Thursday 1 July	8 credits	4 credits

Delegates who are eligible for CPD points should complete the CPD Certificate Request form which will be provided in delegate packs at the meeting.

TRADE EXHIBITION [FOYER]

Delegates are encouraged to visit the Trade Exhibition and are requested to support the companies represented there.

Fees and Registration

REGISTRATION FEES FEES INCLUDE REFRESHMENTS AND LUNCH				
DELEGATE TYPE	FEE CATEGORIES	DAY or PART DAY		SOCIETY DINNER
		UP TO AND INCLUDING 7 JUNE 2010	AFTER 7 JUNE 2010	
Pathological Society Members	Ordinary Members, Consultant and/or equivalent position	£ 90	£ 140	£ 50
Pathological Society Concessionary Members	Biomedical Scientists; Honorary or Senior Members; PhD Students; Post-Doctoral Fellows, Technicians and Trainees	£ 30	£ 50	£ 50
Undergraduate Students ❇		£ 30	£ 50	£ 50
Non-Members	Consultant and/or equivalent position	£ 140	£ 200	£ 50
Non-Members Concessionary ❇	Biomedical Scientists; PhD Students; Post-Doctoral Fellows, Technicians and Trainees	£ 45	£ 70	£ 50

ADVANCE REGISTRATION

Registration is via our on-line facility found on our website: **www.pathsoc.org**
Advance registration will close at midnight on Wednesday 16 June 2010.
Thereafter delegates may only register on-site on arrival at the meeting.

❇ CONCESSIONS

Delegates from categories:

Undergraduate Students
Non-Members Concessionary

must provide an identification document as proof of their student or trainee status, including NTN's where applicable. Proof must be by way of a statement from the Head of Department.
A template document is available on our website: **www.pathsoc.org**
Please e-mail documents to: **julie@pathsoc.org** — or fax to: +44 (0)20 7930 2981.

CANCELLATIONS

Please note that a cancellation fee of £20 will be deducted from any refund due for cancellations received in writing by Monday 7 June 2010. Thereafter a 25% charge will be made for cancellations received in writing before Monday 14 June 2010, thereafter no refunds will be made.

DELEGATE ENROLMENT (AT THE MEETING)

Enrolment at the **Delegate Reception Desk** will take place on:

Tuesday 29 June from 09.00
Wednesday 30 June from 08.00
Thursday 1 July from 07.30

PRESENTATIONS

Presentation Checking and Preview [COMPUTER ROOM – GROUND FLOOR]

Oral Presentations/Lectures – Presentation format

Powerpoint Only (must be PC compatible)
Must be on memory sticks only
Presenters must attend their nominated lecture theatre 30 minutes before their presentation time

INTERNET ACCESS [COMPUTER ROOM – GROUND FLOOR]

Internet access will be available.

MESSAGES

During the Meeting, messages for delegates may be left at the following telephone number: **(0)7818 640887**
There will also be a message board located beside the Registration Desk.

REFRESHMENTS

All refreshments will be served in the Foyer unless stated otherwise in the programme.

BADGES

Delegates are requested to wear their badges **at all times**.

COATS AND BAGS [LOCATION – *TO BE ARRANGED*]

Secure facilities will be provided for coats and bags.

SOCIAL ACTIVITIES

Tuesday 29 June	18.30–20.00	**Welcome Reception** Parliament Hall, University of St Andrews. *Please reserve your free ticket when registering.*
Thursday 1 July	19.30–23.00	**Society Dinner** Lower College Hall, University of St Andrews. *Please reserve your ticket (cost £50) when registering – places are limited.*

LOCAL PLACES OF INTEREST
Visit this website for information: **www.visit-standrews.co.uk**

DISCLAIMER

The Pathological Society of Great Britain & Ireland cannot be held responsible for any injury or loss sustained during the Meeting.

ENQUIRIES

Enquiries before the Meeting regarding administration should be directed to:

Pathological Society of Great Britain & Ireland
2 Carlton House Terrace, London, SW1Y 5AF
Tel: +44 (0)20 7976 1260
Fax: +44 (0)20 7930 2981
Email: admin@pathsoc.org

FUTURE MEETINGS

2010	
23–25 August	**Summer School:** Institute of Cancer Research, London. ***Topics in Molecular Pathology: Concepts, Methods and Applications – Lessons from Breast Cancer***
17 November	**Educational Day**, Royal College of Pathologists, London. ***Molecular Pathology and Colorectal Neoplasia – What a diagnostic pathologist should know and do***
2011	
6–7 January	**Winter Meeting**, inc Trainees' Programme, Cambridge
10–13 May	**Ghent Pathology 2011**, Joint Meeting of the British Division of the IAP and the Pathological Society
2012	
5–6 January	**Winter Meeting**, inc Trainees' Programme, London
3–6 July	**Summer Meeting**, including Trainees' Programme, Sheffield

09.00 **FOYER**
REGISTRATION AND COFFEE

COMPUTER ROOM – GROUND FLOOR *(for Virtual Slides)*
ROOM 235 – BETWEEN LECTURE THEATRES Ⓐ AND Ⓑ *(for Microscope Slides)*

10.00–17.30 **SLIDE SEMINAR COMPETITION VIEWING: *Head/Neck/Oral Pathology***

LECTURE THEATRE Ⓐ

10.00–10.15 **OPENING CEREMONY**
Chair: Prof S Fleming, University of Dundee
Prof CS Herrington, University of St Andrews
Speaker: Dr L Richardson, Principal, University of St Andrews

LECTURE THEATRE Ⓐ

10.15–13.00 **SYMPOSIUM: *Cell Signalling***
Chair: Prof S Fleming, University of Dundee

10.15–10.45 [**S1**] ***Hypoxia Signalling and Disease***
Prof P Ratcliffe, Nuffield Department of Medicine, University of Oxford

Studies of the dynamic regulation of the haematopoietic growth factor erythropoietin by oxygen led to the discovery of a widespread system of gene regulation by oxygen that is conserved throughout the animal kingdom. Responses are mediated by an α/β heterodimeric complex termed hypoxia inducible factor (HIF) that binds hypoxia response elements at target genes involved in angiogenesis, energy metabolism, matrix metabolism, pH regulation, cell survival and proliferation decisions. The oxygen sensitive signal that regulates HIF is generated by a series of non-haem Fe(II) and 2-oxoglutarate (2OG) dependent dioxygenases that catalyse the post-translational hydroxylation of specific prolyl and asparaginyl residues in HIF-alpha subunits. HIF prolyl hydroxylation promotes proteolytic destruction of HIF by the von Hippel-Lindau ubiquitin E3 ligase complex whereas HIF asparaginyl hydroxylation reduces HIF activity by blocking interaction with transcriptional co-activators. Since the HIF hydroxylases have an absolute requirement for dioxygen these processes are suppressed in hypoxia, allowing the HIF-α to escape destruction and activate transcription. Dependence on 2-OG, Fe(II) and ascorbate potentially enables the system to integrate hypoxic, redox and metabolic signals. The HIF system is activated in a wide range of human pathologies including neoplasia, ischaemic, hypoxic and inflammatory diseases. Micro-environmental hypoxia plays an important role in activation of HIF in solid tumours and regions of inflammation. The system is also activated even in the presence of oxygen by cross-talk with oncogenic and inflammatory pathways. Actions in the pathogenesis of disease and potential for therapeutic intervention will be discussed.

10.45–11.15 ***Lipids, PPAR Signalling and Cancer***
Prof C Palmer, Biomedical Research Institute, University of Dundee

11.15–11.45 **COFFEE [FOYER]**

11.45–12.15 [**S2**] ***Ras, Cell Signalling and Mouse Models of Cancer***
Dr MJ Arends, University of Cambridge, Addenbrooke's Hospital

Mutational activation of the K-ras oncogene occurs in ~30% of human adenocarcinomas, in particular in adenocarcinomas of lung (30%), pancreas (90%) and colon (40%). In colorectal carcinogenesis there is cellular transit through a spectrum of neoplastic changes, from microadenomas to adenomas with increasing dysplasia to adenocarcinoma. Some of the transitions are associated with characteristic genetic changes that occur at a relatively high frequency, including alterations to APC (~80%), K-ras (~40%), and the DNA mismatch repair (MSH2 or MLH1) genes (~15%), amongst others. K-ras mutations are found more often in adenomas with increasing size and dysplasia. Valine mutation at codon 12 of the K-ras gene is associated with a poorer prognosis for colorectal cancers, suggesting that it confers aggressive growth properties in carcinogenesis. Activating K-ras mutations at codons 12, 13 & 61 affect GAP-binding and reduce the protein's intrinsic GTPase activity, holding it in an active GTP-bound conformation that produces increased signalling to downstream effectors such as the RAF-MAPK and PI3K/Akt pathways and others. Mouse models with activation of expression of mutant K-ras in the small and large intestines have shown that K-rasV12 cooperates with either mutated Apc or inactivated Msh2 to accelerate intestinal adenoma formation and this involves activation of both the RAF-MAPK and PI3K/Akt pathways. Similarly, a K-rasD12 conditional model recapitulates the premalignant changes of pancreatic and oral carcinogenesis. Knockout of K-ras exon 4A has provided new insights into the roles of the K-ras 4A and 4B isoforms in both lung and colonic tumour formation.

12.15–12.45 [**S3**] ***PTEN and Cancer***

Dr N Leslie, University of Dundee

The phosphoinositide 3-kinase (PI3K) signalling pathway plays an evolutionarily conserved role in controlling cell growth, proliferation and survival in many cell lineages. It seems likely that this is a key reason why a large proportion of all human tumours display increased activity of components of the PI3K signalling pathway. In particular, activating mutations in the PI3K catalytic subunit, p110alpha, and loss of function mutations of the opposing PTEN phosphatase are amongst the most frequent genetic changes in human cancers. Also, several novel candidate tumour suppressors and oncogenes have been identified recently which appear to affect tumour development principally via effects on PTEN. Our work aims to understand how loss of PTEN function and regulation contribute to tumour development. Currently, we are attempting to address the significance of PTEN's dual specificity for both phosphoinositide lipids and peptide substrates through the development and use of mutants that selectively metabolise either lipid or protein substrates. These experiments have been conducted using viral re-expression of PTEN at physiological levels in U87MG glioblastoma cells that lack the phosphatase. The data indicate that PTEN's protein phosphatase activity is not required for its control of total cellular levels of the PI3K product lipid, PIP3, or Akt signaling. However, both lipid and protein phosphatase activities appear to act together to affect several downstream responses that include the regulation of invasion and proliferation in 3D matrigel culture models of glioblastoma.

12.45–13.00 [**S4**] ***Activation Status of Receptor Tyrosine Kinase Downstream Pathways in Primary Lung Adenocarcinoma with Reference of KRAS/EGFR Mutations and EML4-ALK Translocations.***

Dr Y Ishikawa, The Cancer Institute, Japan Institute for Cancer Research, Tokyo

Background: The activation status of signal transduction pathways involving receptor tyrosine kinases and its association with EGFR or KRAS mutations in lung cancer are still uncertain in primary tumours. In Japan, about half of lung adenocarcinomas harbor EGFR mutations and about 10% have KRAS mutations, both being mutually exclusive. Materials and methods: We immunohistochemically examined phosphorylated (p-) Akt, pERK and other downstream proteins using surgical samples of 193 primary lung adenocarcinomas (F:M=97:96). Also, TTF-1 expression as a cell lineage marker and mutation status of EGFR/KRAS genes and EML4-ALK translocation were examined. For histology, we employed such invasiveness criteria as pre-, minimally- and overt invasive.. Results: Histologically, 23% of tumours were pre- and minimally-invasive. Immunoreactivity of pAkt, pERK and TTF-1 were 38%, 36% and 80%, respectively. Mutation frequencies of EGFR and KRAS were 58% and 8%, respectively. Advanced tumour stages ($p<0.001$), negative TTF-1 expression ($p<0.001$) and Akt activation ($p=0.015$) were independent and significant poor prognostic markers. TTF-1 expression was associated with never-smoker status ($p=0.013$), pre- or minimally-invasiveness ($p<0.001$) and EGFR mutations ($p=0.004$) as well as with pERK ($p=0.039$) expression. EGFR mutations did not correlated with pAkt and pERK expression, which was different from the results based on cultured cells, while KRAS mutations were solely and significantly linked to ERK activation ($p=0.015$). Results of EML4-ALK will be presented as well. Discussion: We revealed that the cell lineage was important in outcome estimation at any tumour stage while Akt activation was abnormally affected according to the tumour stage regardless of their cell origin. The signal proteins of primary tumours were differently related to EGFR and KRAS mutation status from cultured cells in lung adenocarcinoma.

FOYER

13.00–14.30 LUNCH AND TRADE EXHIBITION

LECTURE THEATRE Ⓒ

13.30–14.30 TRAINEES' SESSION – MEET THE EXPERTS

Chair: Dr I Proctor, University College London

[**S5**] ***Renal Tumour Pathology***

Prof S Fleming, University of Dundee

The WHO 2004 classification of renal tumours built on the preceding Heidelberg-Rochester classification applying the principles of a morphological classification which was also soundly based on our understanding of the genetics of renal tumours. Since 2004 a number of new or variants forms of renal tumours have been recognised. These recent advances will be the subject of this seminar. It has become clear that several different types of renal tumour may be associated with mutation of the fumarate hydratase gene in the HLRCC syndrome. However, there are morphological features which should raise of suspicion of HLRCC. In previous classifications there has been recognition of a low grade form of the collecting duct carcinoma, this has now be reclassified as tubulocystic carcinoma although its relationship with collecting duct carcinoma remains to be explored fully. Papillary forms of oncocytic tumours have been convincingly shown to be a distinct entity. I will make recommendations for the use of immunocytochemistry and molecular or cytogenetic investigations in histopathology practice for renal tumour diagnosis. The diagnostic criteria and clinical significance of these new renal tumours will be presented in a discussion format.

FOYER

14.30–15.30 POSTER VIEWING AND CHAIRMAN'S ROUNDS

CATEGORY	POSTER NUMBERS
Autopsy & Forensic	P01–P02[1]
Breast	P03–P09[2]
Cardiovascular/Pulmonary	P10–P13[1]
Head & Neck	P14–P17[3]
Lymphoreticular	P18–P26[3]
Neuropathology/Ophthalmic	P27[4]
Osteoarticular/Soft Tissue	P28–P29[4]
Skin	P30–P35[4]
Technical Advances	P36–P40[1] *(Note: P39 withdrawn)*

Chair: [1] Prof K Kerr, Aberdeen and Prof GI Murray, Aberdeen
[2] Dr V Speirs, Leeds
[3] Dr J Goodlad, Edinburgh
[4] Dr N Kirkham, Newcastle-upon-Tyne

LECTURE THEATRE Ⓐ

15.30–17.30 ORAL COMMUNICATIONS

Chair: Dr JJ Going, University of Glasgow
Dr V Speirs, Leeds Institute of Molecular Medicine, St James's University Hospital
Category: Breast

15.30–15.45 [**O1**] ***SOX11 and PSMD3 Expression in HER2 Positive Breast Cancer***

Ⓟ Pang, Y[1]; Ball, G[2]; Rakha, E[3]; Powe, D[3]; Caldas, C[4]; Ellis, I[3]; Green, A[1]

[1]University of Nottingham, United Kingdom; [2]Nottingham Trent University, United Kingdom; [3]Nottingham University Hospitals, United Kingdom; [4]CRUK Cambridge Research Institute, United Kingdom

Human Epidermal Receptor 2 (HER2) positive have attracted attention as a poor prognostic class of breast cancer. However, HER2+ tumours appear to encompass biologically and clinically heterogeneous tumours. In order to refine HER2+ breast cancer, we analysed over 48,000 gene transcripts in 132 invasive breast carcinomas using Artificial Neural Network analysis and identified high expression of two novel genes (PSMD3 located 17q21.1; SOX11 located on 2p25.2,) significantly associated HER2+ positivity. Using a large invasive breast carcinoma cohort (n=1,298), prepared as tissue microarrays, we assessed the protein expression of these targets using immunohistochemistry and investigated their associations with clinicopathological variables, patients' outcome and ability to refine HER2+ classification. PSMD3 nuclear expression was observed in 219/942 (23%) of tumours and was significantly correlated to HER2 positivity (p=0.004), tubule formation (p=0.047) and poor NPI (p=0.007). PSMD3 expression conferred a strong trend towards a longer breast cancer specific survival in the whole series (p=0.065). SOX11 nuclei staining was observed in 96/869 (3.8%) tumours and was significantly associated with PSMD3 nuclear (p<0.001) and ER positivity (p=0.006) and ck14 negativity (p=0.018) but not HER2. SOX11 expression did not predict patient clinical outcome in either the whole series or in the HER2+ tumours only. This study confirms the biological and clinical heterogeneity of HER2+ tumours and the difficulties in translating global gene expression data into routine practice using immunohistochemistry. We have identified two novel genes associated with HER2+ tumours and further studies analysing the role of PSMD3 expression in this important subtype is warranted.

15.45–16.00 [**O2**] ***Differential Expression of CD 151 in DCIS and LCIS: Implications for its Role in Disease Progression***

Ⓟ Romanska, H[1]; Chaudhri, S[1]; Dawoud, M[2]; Jones, L[2]; Potemski, P[3]; Connoly, C[4]; Novitskaya, V[4]; Berditchevski, F[4]

[1]Department of Pathology, University of Birmingham, United Kingdom; [2]Bart's and The London, Queen Mary's School of Medicine and Dentistry, United Kingdom; [3]Department of Pathology and Chemotherapy Medical University of Łódź, Poland; [4]Cancer Research UK Institute for Cancer Studies, United Kingdom

Our recent experimental data suggest that tetraspanin CD151 controls in vivo and in vitro growth of human mammary epithelial cells. To establish physiological relevance of pro-proliferative and morphogenetic activities of CD151 we examined expression of this tetraspanin in breast carcinoma (BCa), with special emphasis on in situ BCa, a hyperpfoliferative precursor to its infiltrating form. We analysed 95 cases of BCa containing both in situ and invasive components (48 ductal and 47 lobular BCa) using immunohistochemistry (IHC). In ductal carcinoma in situ (DCIS), IHC for CD151 showed high immunoreactivity (IR) at the periphery but not in the centre of the duct

and there was a significant association between the level of CD151 expression and the grade of DCIS (p=0.004). In contrast, in lobular carcinoma in situ (LCIS), CD151 was strongly and uniformly expressed by all cells of the lesion, regardless of the tumour characteristics or its expression in the associated infiltrating component. As CD151 forms complexes with laminin-binding integrin α3β1 and via this association regulates tumour cell migration and invasion, we further examined expression of α3 integrin subunit in both LCIS and invasive (ILC) lobular carcinoma. The results demonstrated that there was a significant correlation (p=0.037) between the expression of α3β1 in ILC and in accompanying LCIS. Thus, co-expression of CD151 and integrin α3 in LCIS seems to be highly suggestive of the pattern of their expression in corresponding ILC. These results strongly imply that CD151 may play an important role in the development of hyperproliferative neoplastic diseases in the mammary gland.

16.00–16.15 [**O3**] ***Prognostic Significance of Delta-like Ligand 4 (Dll4) Expression in Breast Cancer***

Ⓟ Jubb, A[1]; Soilleux, E[2]; Turley, H[2]; Steers, G[2]; Parker, A[3]; Low, I[3]; Blades, J[2]; Li, J[2]; Allen, P[3]; Leek, R[2]; Noguera-Troise, I[4]; Gatter, K[2]; Thurston, G[4]; Harris, A[2]

[1]University of Oxford, Department of Clinical Laboratory Sciences, United Kingdom; [2]University of Oxford, United Kingdom; [3]John Radcliffe Hospital, Oxford, United Kingdom; [4]Regeneron Inc, Tarrytown, United States

Purpose: Dll4 is a Notch ligand, predominantly expressed by endothelium. Evidence from xenografts suggests that Dll4 expression may confer resistance to anti-VEGF therapy and inhibiting Dll4 may overcome resistance to anti-VEGF therapy. The aims of this study were to characterize the expression of Dll4 in breast cancer and assess whether it is associated with inflammatory markers and prognosis. Experimental Design: Two hundred and ninety six breast adenocarcinomas and 38 ductal carcinoma in situ tissues were represented in tissue microarrays. Additional whole sections representing 10 breast adenocarcinomas, 10 normal breast tissues and 16 angiosarcomas were included. Immunohistochemistry was performed using validated antibodies against Dll4, CD68, CD14, DC-SIGN, CD123, neutrophil elastase, CD31 and carbonic anhydrase 9. Isotopic in situ hybridization was also performed on a subset of cases to validate Dll4 expression. Results: Dll4 was selectively expressed by tumour-associated endothelial cells in 73 to 100% of breast adenocarcinomas, 18% of in situ ductal carcinomas and all lactating breast cases, but not normal nonlactating breast. High intensity of endothelial Dll4 expression was a statistically significant adverse prognostic factor when compared to no expression of endothelial Dll4 in univariate (hazard ratio 2.29, 95% confidence intervals 1.37 to 3.82, p=0.002) and multivariate analyses (hazard ratio 1.55, 95% confidence intervals 1.05 to 2.29 p=0.03) of overall survival. The inflammatory markers were not significant prognostic factors in multivariate analyses of overall survival. Conclusions: Dll4 is expressed by tumour-associated endothelium. In these retrospective subset analyses, high intensity endothelial Dll4 expression was a statistically significant multivariate prognostic factor.

16.15–16.30 [**O4**] ***Translational Exploration of Akt Pathway Activation in Early Invasive Breast Cancer: Clinicopathologic and Molecular Correlations***

Ⓟ Aleskandarany, M[1]; Green, A[1]; Rakha, E[2]; Ahmed, M[1]; Powe, D[1]; Ellis, I[1]

[1]School of Molecular Medical Sciences, Nottingham, United Kingdom; [2]Department of Pathology Nottingham University Hospitals NHS Trust, United Kingdom

Akt/PKB serine/threonine kinase is a leading signalling mediator involved in the modulation of several cellular processes including cell growth, proliferation, and survival. However, the complexity and diversity in the upstream/downstream arms of Akt pathway, as reported in recent genetic studies, challenge considerably the clinical

Correlations between pAkt expression and the expression of tissue markers	Significance (p value)
ER	0.001
PgR	0.364
AR	0.003
HER2	0.226
EGFR	0.585
HER3	0.216
HER4	0.032
HER2/HER family combinatorial subgroups	0.220
p53	0.039
CK5/6	0.014
CK14	0.236
CK18	< 0.001
CK19	<0.001
E-cad	0.432
P-cad	0.109
PIK3CA	0.006
MIB1	0.011
PTEN	0.033
p21	0.765
p27	0.089
Bcl-2	0.136
Mdm2	0.328
Molecular phenotype: Luminal-like, HER2 Positive, Triple Negaitve, Basal-like BC	< 0.001

evolvement of effective targeted therapies. This study aims to study the expression of phospho-Akt1 (pAkt) in breast cancer (BC), with respect to different component proteins upstream/downstream of Akt pathway activation, and its correlation with clinicopathologic parameters, and disease outcome. pAkt (ser473) was evaluated by immunohistochemistry, on tissue microarrays containing 1,202 early invasive BC, with long term follow-up. pAkt was overexpressed in 76% of BC and its expression was positively associated with estrogen and androgen receptors, PIK3CA, cytokeratin (CK)18, CK19 and PTEN expression. Loss of pAkt was correlated with higher grade, CK5/6, p53 and Ki-67 labelling index. Luminal-like tumours displayed more pAkt positivity than triple negative/basal-like subtypes. pAkt expression was not significantly associated with breast cancer specific (BCSS) or metastasis free survival (MFS). Interestingly, sustentative proportions of tumours were PIK3CA-/pAkt+ or PIK3CA+/pAkt- when its expression was considered in combination with PIK3CA, and the resulting 4 phenotypes were significantly associated with BCSS (p=0.001) and MFS (p=0.002). Although pAKT is an oncogene that correlated with poor prognostic variables, its expression is not a prognostic marker. Combinatorial phenotypic groups of PIK3CA/pAkt denoted functional complexity, at translational level, within the upstream and downstream arms of Akt activation with significant impact on patients' outcome. These findings may help determine more adequate therapeutic regimens for specific subgroups of this key cancer pathway.

16.30–16.45 [**O5**] ***Candidate Molecular Markers as Correlates of the Basal Phenotype in Breast Cancer***

Ⓟ Baker, B[1]; Ball, G[2]; Rakha, E[3]; Powe, D[3]; Caldas, C[4]; Ellis, I[3]; Green, A[1]

[1]University of Nottingham, United Kingdom; [2]Nottingham Trent University, United Kingdom; [3]Nottingham University Hospitals, United Kingdom; [4]CRUK Cambridge Research Institute, United Kingdom

Basal-like cancers (BP) have attracted attention as a poor prognostic class of breast cancer. However, BP appear to encompass biologically and clinically heterogeneous tumours. In order to refine BP definition, we analysed 48,000 gene transcripts in 132 invasive breast carcinomas using ANN analysis and identified two novel genes whose expression PPARα (positive) and GMPR2 (negative) was significantly associated with BP (cytokeratin(CK)5/6 and/or CK14 positive). Using a large invasive breast carcinoma cohort (n=1,200), prepared as TMAs, we assessed these targets immunohistochemically and investigated associations with clinicopathological variables, patients' outcome and ability to refine BP classification. Lack of PPARα and GMPR2 protein expression was associated with basal phenotype (p=0.023, p=0.001 respectively). Positive expression of both markers were associated ER and PgR positive status (p<0.05) and with the good prognostic NPI group (p=0.012, p<0.001 respectively). Lack of PPARα expression was associated with a shorter disease-free interval (p=0.047), and GMPR2 with a shorter breast cancer specific survival (p=0.007) but these were not independent of size, stage and grade. In this study, we have identified GMPR2 and PPARα as novel biomarkers which have anassociation with outcome, clinicopathological parameters and biomarkers of prognostic value in breast cancer. Negative expression of these markers is associated with BP and loss of their expression may play a role in carcinogenesis of this molecular subtype. Therefore further studies into their relevance in further classification of BP are warranted.

16.45–17.00 [**O6**] ***Impact of Exploratory Biomarkers on the Treatment Effect of Bevacizumab in Metastatic Breast Cancer***

Ⓟ Jubb, A[1]; Hillan, K[2]; Koeppen, H[2]

[1]University of Oxford, Department of Clinical Laboratory Sciences, United Kingdom; [2]Genentech Inc, South San Francisco, United States

Purpose: The addition of bevacizumab to chemotherapy has a proven progression-free survival (PFS) benefit in advanced/metastatic breast cancer (MBC). However, the addition of bevacizumab to capecitabine failed to demonstrate a survival benefit in patients with previously treated MBC (AVF2119g trial). The aim of these retrospective subset analyses was to evaluate the expression of novel putative biomarkers as predictors of benefit from bevacizumab. Patients and Methods: In the AVF2119g trial, 462 patients with MBC were randomly assigned to receive capecitabine or capecitabine plus bevacizumab. Primary tumour tissue and outcome data were available for 223 patients. Biomarker expression was assessed by in situ hybridization (VEGF-A, VEGF-B, thrombospondin-2 and Flt4) or immunohistochemistry (VEGF-C, PDGF-C, neuropilin-1, delta like ligand (Dll)4, Bv8, p53 and thymidine phosphorylase) on archival tissue. PFS was associated with these variables in retrospective subset analyses. Results: Patients with low scores for Dll4, VEGF-C, neuropilin-1 and thymidine phosphorylase showed trends towards improvement in PFS associated with the addition of bevacizumab to capecitabine (p values 0.01, 0.05, 0.05 and 0.07, respectively). However, the data were not statistically significant after correction for multiple hypothesis testing. Conclusion: These retrospective subset analyses, suggest that expression of angiogenesis factors may predict benefit from bevacizumab. Such observations are not conclusive, but warrant additional testing.

17.00–17.15 [**O7**] ***Molecular and Clinicopathological Correlates of Breast Cancer Growth***

Ⓟ Wormall, A; Aleskandarany, M; Burell, H; Luck, A; Doyle, S; Powe, D; Ellis, I; Green, A; Rakha, A

Nottingham University, United Kingdom

Information on breast cancer (BC) growth rate has many medicolegal, biological and clinical implications contributing to patients' management decision. The growth rate of breast cancer (BC) in vivo is influenced mainly by primary tumour biological factors such as balance between growth fraction and apoptosis. This study aimed to assess the molecular and clinicopathological determinants of BC growth rate in vivo. The study group were BC

diagnosed in women who participated in a screening program with serial mammography available (n = 105). The growth rate, expressed as the tumour volume changes over specific time intervals was determined. Expression of the proliferation marker Ki67, apoptosis marker cleaved Caspase-3, ER, PgR, HER2 and the basal marker Cytokeratin 5/6 were determined by immunohistochemistry. Growth rate was correlated with these biomarkers and pathological parameters in order to determine the factors that influence BC growth rate. Results: Overall, change in size between screening and diagnosis in each patient varied significantly; 5 cases showed no change and one case showed tumour shrinkage. Growth rate ranged from -0.43-2.45 mm/month (mean=0.55, median=0.43mm/month). A highly significant correlation was found between higher tumour growth rate and tumour grade (p=0.012), mitotic index (p<0.001), tumour size at the time of diagnosis (p<0.001), Ki67 expression (p=0.013) and presence of vascular invasion (p=0.014). There was no significant relationship between growth rate and LN stage, tumour size at the time of initial screening, or expression of ER, PgR, HER2, or caspase-3. Conclusion: Assessment of primary tumour histologic grade, mitotic counts and ki67 expression can be used as a predictor of in-vivo tumour growth rate. The role of proliferation rather than apoptosis seems to be the main determinant of breast cancer growth rate. Further confirmatory studies are warranted.

17.15–17.30 [**O8**] ***Oestrogen Receptor Positive Breast Cancer: Prognostic and Biological Significance of Proliferation Assessed by Cyclin B1 and Thymidine Kinase 1 (TK1) Protein Expression***

Ⓟ Habashy, H[1]; Powe, D[2]; Ball, G[3]; Green, A[4]; Rakha, E[2]; Ellis, I[4]

[1]Department of Pathology, School of Molecular Medical Sciences, University of Nottingham, Queen's Medical Centre, United Kingdom; [2]Department of Cellular Pathology, Nottingham University Hospitals NHS Trust, United Kingdom; [3]School of Science and Technology, Nottingham Trent University, United Kingdom; [4]Department of Pathology, School of Molecular Medical Sciences, University of Nottingham, United Kingdom

The importance of cellular proliferation in subclassifying breast cancer (BC) is recognised. We tested if cell cycle phase specific protein expression can identify BC prognostic subclasses especially in the oestrogen receptor (ER) positive luminal-like subtype. TK1 is an enzyme involved in the synthesis of thymidine triphosphate needed by the proliferating cells to enter S phase and Cyclin B1 is essential for the initiation of mitosis as it regulates the G2-M transition of the cell cycle. In this study, we explored the clinical and biological relevance of TK1 and Cyclin B1 protein expression in a large well-characterised series of patients presenting with operable stage I-III BC (n=1902) using tissue microarrays and immunohistochemistry. The data on other biomarkers with strong relevance to ER positive breast cancer were available. Expression of either TK1 or Cyclin B1 showed positive associations with markers of poor prognosis including high histologic grade, lymph node stage, development of distant metastasis and tumour recurrence. They also showed positive associations with the expression of p53, HER2, p-cadherin and the pan-proliferation marker MIB1. TK1 expression were inversely associated with steroid receptors, Bcl2 and luminal cytokeratins expression. In the luminal-like cohort, they showed some similar associations and were associated with shorter breast cancer specific survival and distant metastasis free interval (p<0.05). Patients co-expressed both markers showed shorter survival (p=0.006) than those expressing an individual protein. In conclusion, overexpression of Cyclin B1 and TK1 showed a correlation with more aggressive phenotype in BC particularly in the luminal-like cancers and has a potential use for stratifying this heterogeneous group. Our findings support previous studies that targeting their expression in high proliferative BC provides an important treatment approach.

LECTURE THEATRE Ⓑ

15.30–17.30 ORAL COMMUNICATIONS

Chair: Dr J Goodlad, Western General Hospital, Edinburgh
Dr P Johnston, Aberdeen Royal Infirmary

Categories: Lymphoreticular; Osteoarticular/Soft Tissue; Autopsy/Forensic; Education and Audit

15.30–15.45 [**O9**] ***Examination of a Panel of Tumour-Associated Macrophage Genes in DLBCL Indicates that LAIR-1 Expression Patterns are Associated with Outcome.***

Ⓟ Doig, T[1]; Sieniawski, M[2]; Freeman, T[3]; Gregory, C[1]; Goodlad, J[4]

[1]Centre for Inflammation Research, Edinburgh, United Kingdom; [2]Scottish and Newcastle Lymphoma Group, Newcastle, United Kingdom; [3]The Roslin Institute, Edinburgh, United Kingdom; [4]Lothian University NHS Trust, Edinburgh, United Kingdom

Background: Tumour-associated macrophages are hypothesised to play key roles in tumour growth including immunosuppression, support of tumour growth, angiogenesis and promotion of metastasis. Data from large gene expression studies of Diffuse Large B cell lymphoma (DLBCL) suggest that genes expressed in the microenvironment are predictive of prognosis, but the role of the tumour-associated macrophage has not been elucidated. Methods: In this study a gene expression profile of tumour-associated macrophages in DLBCL was derived from a large human data set using the analysis tool BiolayoutExpress3D. A panel of markers of tumour–associated macrophages from this profile was selected and expression was assessed by immunohistochemistry in an independent cohort of 80 cases of DLBCL treated with CHOP, identified from the SNLG database, and effects on outcome examined. Results and conclusions: In this cohort, expression of TYMP, AIF1 and LGALS3BP by tumour associated macrophages did not predict outcome, but patients with low expression of LAIR1 showed higher rates of

overall survival compared to those with high expression levels (Chi-squared test, p<0.05). LAIR 1 is an inhibitory immunoglobulin-like molecule expressed widely in the immune system, which binds collagen in the extracellular matrix. The role of this molecule in the tumour microenvironment has not yet been studied and merits further investigation.

15.45–16.00 [**O10**] ***Identification of Pathological Markers of MYC Translocation in Large B-cell Lymphoma.***

Ⓟ Kirkwood, K[1]; Hart, A[2]; Hendry, L[3]; Day, F[3]; Bauld, R[3]; Raffan, M[3]; Krajewski, A[1]; Goodlad, J[1]

[1]Department of Pathology, Western General Hospital, Edinburgh, United Kingdom; [2]Department of Haematology, Western General Hospital, Edinburgh, United Kingdom; [3]South East Scotland Cytogenetics Laboratory, Western General Hospital, Edinburgh, United Kingdom

B-cell lymphomas with large cell morphology constitute a heterogeneous group of disorders with varying responses to therapy. Most are currently classified as diffuse large B-cell lymphoma (DLBCL) on the basis of pathological examination. However, translocations involving the MYC gene identify certain large B-cell lymphomas (LBCL) with aggressive clinical behaviour. These MYC translocated tumours are currently considered for more aggressive chemotherapy regimens than typical DLBCL, but are often indistinguishable from them on the basis of routine examination. FISH analysis is a sensitive means of identifying specific translocations in formalin fixed tissues but is costly, time consuming and not yet part of routine practice. In this study we set out to determine whether we could identify any pathological features that correlated with the presence or absence MYC translocation, in order to facilitate future targeted practice.
FISH analysis was performed prospectively on 73 unselected cases LBCL using dual colour breakapart probes. Hybridization for MYC was done on all cases and, if positive, was followed by hybridization for BCL2 and BCL6. MYC rearrangement was found in 18 cases, 7 of which had a second abnormality. The presence of MYC translocation correlated positively with high Ki67 index (p<0.001), positive staining in tumour cells for CD10 (p=0.005) and tumour cell expression of Bcl6 (p=0.03). MYC rearrangement was not significantly associated with Bcl2, MUM1 or CD5. However, the proportion of cases expressing BCL2 protein was significantly lower in MYC only translocation cases compared to those with additional abnormalities (p<0.01).
Pathological assessment for CD10, Bcl6 and Ki67 in cases of LBCL is quick, easy and may provide a relatively inexpensive method for indentifying cases which could benefit from further FISH analysis.

16.00–16.15 [**O11**] ***Molecular Diagnosis of Mantle Cell Lymphoma in Formalin Fixed Paraffin Embedded Samples: A Comparison of FISH and PCR Techniques***

Ⓟ Majeed, W[1]; Diss, T[2]; Ye, H[3]; Ramsay, A[2]; Malagoli Rocha, R[4]

[1]University College London Hospital, Histopathology Department, United Kingdom; [2]University College London Hospital, United Kingdom; [3]Royal National Orthopaedic Hospital, Stanmore, United Kingdom; [4]A.C. Camargo, São Paulo, Brazil

The diagnosis of mantle cell lymphoma (MCL) can be difficult using conventional microscopy and immunohistochemistry in a proportion of cases. These may be resolved using molecular techniques that target hallmark MCL chromosome translocation t(11;14) which has also been detected in plasmacytomas (PC). The most widely used techniques are fluorescence in-situ hybridisation (FISH) and polymerase chain reaction (PCR). However, these have not been fully evaluated in routine formalin fixed paraffin embedded (FFPE) tissue samples that may have relatively poor DNA preservation. 18 MCL, 15 PC and 19 follicular lymphomas (FL) were studied by FISH using break-apart CCND1 and dual fusion CCND1/IGH probes and by PCR amplification of the major translocation cluster/IGH region. PCR was performed using standardised Euroclonality/Biomed-2 primers, an in-house method designed to target smaller fragments, and a real-time PCR approach designed to improve sensitivity. DNA quality was assessed by multiplex PCR amplification of 100-400bp fragments. 17 of 18 (94%) MCL were positive using FISH with both break-apart and dual fusion probes. 1 PC and no FL were positive with FISH. Three cases of MCL (17%) were positive using PCR irrespective of primer set or methodology adopted. All PC and FL were negative by PCR. DNA quality was variable and generally poor with 31/52 cases unable to support amplification of fragments greater than 200bp. However PCR positivity was not related to better DNA quality. These data show that detection of t(11;14) in FFPE material is best carried out using FISH, which seems to be little compromised by poor DNA quality. The implementation of a range of PCR primer sets targeting the MTC region of CCND1 failed to improve detection rates, suggesting that breakpoints frequently occur outside the MTC.

16.15–16.30 [**O12**] ***Apoptosis in Follicular Lymphoma Divided by t(14:18)(q32;q21) Status***

Ⓟ Doig, T[1]; Marjenberg, D[2]; Dunbar, D[1]; Sales, M[3]; Pratt, N[3]; Batstone, P[4]; Goodlad, J[5]

[1]Queen's Medical Research Institute, Edinburgh, United Kingdom; [2]Agilent, Dundee, United Kingdom; [3]Ninewells Hospital, Dundee, United Kingdom; [4]Aberdeen Royal Infirmary, United Kingdom; [5]Lothian University NHS Trust, Edinburgh, United Kingdom

In follicular lymphoma, the characteristic t(14:18)(q32;q21) translocation resulting in deregulated expression of bcl-2 is seen in the majority of cases. A subset of cases, accounting for 10-15% of the total, lack this translocation suggesting factors other than a bcl-2-promoted resistance to apoptosis may play a role in these cases. A previous study demonstrated few genetic differences between follicular lymphoma with and without the translocation. We studied the expression of bcl-2 family proteins in these cases to establish whether the role of bcl-2 in inhibiting apoptosis is substituted for by another member of this family in t(14:18)(q32;q21) negative tumours.

Methods: Expression of bcl-2 family members, proliferative index and apoptotic index were assessed by immunohistochemistry on a tissue microarray of follicular lymphoma (constructed by Tayside Tissue Bank).
Results: Absence of the t(14:18) translocation was confirmed by classical cytogenetics, FISH and PCR in 11 of 93 (12%) cases of follicular lymphoma of grades 1-3A . The expression of bcl-2 was present in significantly more cases with the t(14:18) translocation than those without (ANOVA p=0.0001). There were no significant differences in expression of other bcl-2 family members analysed between subtypes (bcl-xl, bid, bax). Additionally, neither the proliferative index nor the apoptotic index differed significantly between subsets.
Discussion: These findings show that bcl-2 protein was not expressed in most cases of follicular lymphoma of grade 1-3A lacking the t(14;18) translocation and that there was no upregulation of other anti-apoptotic proteins to compensate since no differences were found in apoptotic index between t(14:18) negative and positive cases, suggesting that additional mechanisms other than bcl-2 mediated resistance to apoptosis may play a role in these tumours.

16.30–16.45 [**O13**] ***In Vivo and In Vitro Implications of the Transcription Factor T (Brachyury) in Pathogenesis of Sporadic Chordomas***

Ⓟ Shalaby, A[1]; Presneau, N[2]; Pillay, N[2]; Ye, H[3]; Idowu, B[3]; Halai, D[2]; Tirabosco, R[3]; Jacques, T[4]; Mertens, F[5]; Szuhai, K[6]; Kindblom, L[7]; Hogendoorn, P[8]; Flanagan, A[3]

[1]Institute of Orthopaedics and Musculoskeletal Science, University College London, United Kingdom; [2]Cancer Institute, University College London, United Kingdom; [3]Department of Histopathology, Royal National Orthopaedic Hospital, United Kingdom; [4]Neural Development Unit, UCL ICH and Department of Histopathology, G Ormond Street Hospital, United Kingdom; [5]Department of Clinical Genetics, Lund University Hospital, Sweden; [6]Department of Molecular Cell Biology, Leiden University Medical Center, Netherlands; [7]Department of Musculoskeletal Pathology, Royal Orthopaedic Hospital, Stanmore, United Kingdom; [8]Department of Pathology, Leiden University Medical Center, Netherlands

Chordomas are rare primary malignant bone tumours that express brachyury, a gene uncommonly expressed in other tumours and crucial for notochord development. In a recent study of familial chordomas brachyury duplication was found to confer a major susceptibility in four of seven studied families. AIM: to investigate whether brachyury copy number was increased in sporadic chordomas and to study the effect of brachyury silencing on U-CH1 cell line, a bona fide chordoma-derived cell line with which we have established a xenograft showing typical chordoma morphology and immunophenotype. METHODS: A combination of quantitative-PCR, FISH and array-CGH were used to investigate a series of 156 chordomas (sacro-coccygeal (n=86), skull-based (n=58) and mobile spine (n=12)). A lentiviral vector expressing brachyury-targeting shRNA was used to knockdown brachyury in U-CH1 cells. RESULTS: 72 of 156 chordomas (46%) analysed by at least two technologies in most cases, revealed chromosomal abnormalities involving brachyury locus, none of which were germline. These included amplification (7/156, 4.5%, with one case showing further copy number gain on recurrence), chromosome 6 aneuploidy (49/153, 32%), and brachyury locus imbalance (15/138, 11%). One case showed aneuploidy in addition to insertion of the brachyury locus into chromosome 2q. The knockdown of brachyury revealed a marked decrease in cell proliferation and striking morphological changes consistent with premature senescence. CONCLUSION: Genetic abnormalities involving brachyury are common in sporadic chordomas and these along with our in vivo and in vitro data provide strong evidence that brachyury is crucial in the pathogenesis of this disease.

16.45–17.00 [**O14**] ***Epidermal Growth Factor Receptor as a Potential Therapeutic Target in Chordomas***

Ⓟ Shalaby, A[1]; Ye, H[2]; Diss, T[3]; Whitwell, D[4]; Jacques, T[5]; Tirabosco, R[2]; Presneau, N[6]; Flanagan, A[2]

[1]Institute of Orthopaedics and Musculoskeletal Science, UCL, Middlesex, United Kingdom; [2]Department of Histopathology, Royal National Orthopaedic Hospital, United Kingdom; [3]Department of Histopathology, University College London Hospitals, United Kingdom; [4]Nuffield Orthopaedic Centre, Oxford, United Kingdom; [5]Neural Development Unit, UCL ICH and Department of Histopathology, Great Ormond Street Hospital, United Kingdom; [6]Cancer Institute, University College London, United Kingdom

Chordoma is a rare malignant bone tumour with a high risk of recurrence and considerable metastatic potential. Currently, chordoma has no effective drug therapy, although there is evidence that some patients respond to empirical use of EGFR antagonists.
AIM: to analyse the expression and genetic changes of epidermal growth factor receptor (EGFR) in chordomas and to determine the effect of EGFR inhibitors on U-CH1 chordoma-derived cell line.
METHODS AND RESULTS: Tissue microarrays of 50 non skull-based and 50 skull-based chordomas were analysed by immunohistochemistry and revealed EGFR expression in 66% and 62.5% respectively. FISH analysis failed to reveal EGFR amplification; however 45% of the cases and the U-CH1 cell line showed high level polysomy (more than or equal to 4 copies/cell in more than or equal to 40% of cells); 18% - low level polysomy (more than 2 copies/cell in less than or equal to 40% of cells or 3 copies/cell in more than or equal to 40% of cells); 38% - disomy (less than or equal to 2 copies in more than 90% of the cells). Phospho-RTK array membranes showed EGFR activation in U-CH1 cells and the three chordomas analysed. No mutations were detected by direct sequencing of exons (18-21) encoding the EGFR kinase domain in 23 cases. The EGFR inhibitor (AG1478) markedly inhibited the U-CH1 cell proliferation and diminished the EGFR phosphorylation in a dose-dependent manner (5-200 nmol/L) without affecting brachyury expression.
CONCLUSION: Successful treatment of chordomas with anti-EGFR agents may depend on the level of EGFR

aneusomy and warrants clinical testing. Failure of chordomas with high level aneusomy to respond to EGFR antagonists will warrant screening for mutations of the RAS-MAPkinase downstream of EGFR.

17.00–17.15 [**O15**] ***Rapidly Fatal CD8+ T-cell Encephalitis in African HIV+ve Patients on Effective Long-Term Combination Anti-Retroviral Therapy: a New Clinico-Pathological Entity in AIDS?***

Lucas, S[1]; Ⓟ Bhagwat, P[2]; Bridges, L[2]; Mahadeva, U[3]

[1]KCL School of Medicine, London, United Kingdom; [2]St George's Hospital, London, United Kingdom; [3]St Thomas' Hospital, London, United Kingdom

Introduction. HIV brain pathology includes a specific HIV encephalitis (white matter multi-nucleate giant cell nodules, HIVp24 antigen positive). Since the introduction of combination anti-retroviral therapy (cART) in 1997, an immune reconstitution inflammatory syndrome (IRIS) has been recognised: the brain pathology is a pre-dominant CD8+ T-cell perivascular and parenchymal encephalitis, no HIVp24. We present a previously unreported pattern of CD8 brain disease in patients with effective long-term cART. Methods. Review of the HIV pathology files, 2002-10, selecting for CD8+ T-cell encephalitis (CD8TCE); review of the clinical, imaging, blood and CSF CD4 and HIV viral load data, cART treatment history. Results. 7 patients had florid CD8TCE, with no other infection demonstrated, nor HIVp24 antigen in the brain. None had DILS (diffuse infiltrative CD8 lymphocytosis syndrome). All were black African, 5 female, 2 male, median age = 43yrs (range 28-46). 6/7 suffered unexpected unresuscitatable collapse in hospital or community. For 5 patients (group A), the time on cART ranged from 4-13 yrs, with CD4 counts 200-750, and undetectable blood HIV viral loads; neuro-imaging prior to collapse in 4 patients showed diffuse or multi-focal white matter damage. In contrast, 2 patients (group B) had the same clinical pathology, but one was only 7 months on cART with final CD4 count 291 (=IRIS); the other had stopped cART and had a terminal viral load of 868,000 (=reactivation HIV CD8TCE). Conclusion. The five patients in group A do not fulfil the criteria for HIV encephalitis nor for cART IRIS (ie presenting within 6 months). But after years of chronic stable HIV disease, viral load undetectable, they present with encephalitic cerebral oedema and die rapidly, with an abnormal influx of CD8+ T-cells. This pathology is not directly drug-related. African ethnicity suggests a host factor is operating.

17.15–17.30 [**O16**] ***Audit of Lymph Node Yields from Axillary Clearance Specimens in a District General Hospital***

Ⓟ Jubb, A[1]; Chia, Y[2]

[1]University of Oxford, Department of Clinical Laboratory Sciences, United Kingdom; [2]Wycombe Hospital, High Wycombe, United Kingdom

Purpose: The appraisal of lymph node involvement by breast adenocarcinoma has prognostic implications and influences patient management. For this reason the Association of Breast Surgery's minimum standard is a lymph node yield ≥10 in 90% of axillary clearances. The aim of this audit is to assess the lymph node yield from axillary clearance specimens for breast carcinoma at Wycombe Hospital in 2009 and to identify factors associated with poor yields. Methods: The WinPath database was queried using SNOMED codes to identify all axillary clearance specimens received in the Cellular Pathology Department between 01/01/2009 and 31/12/2009. Results: Fifty one axillary clearance specimens were reported in 2009, of which 22 were performed following a positive sentinel lymph node biopsy and 29 were performed at the time of excision of the primary cancer. A mean 15.2 lymph nodes were obtained from each specimen (standard deviation, 5.6; range, 2 to 27). Forty two of 51 specimens (82.4%) were reported to contain ≥10 lymph nodes. Of 9 specimens with <10 nodes, 7 were embedded in their entirety. The remaining 2 cases were both pN1 with yields of 9 nodes. Axillary clearances with node yields <10 had a significantly lower mean weight compared to those with yields ≥10 (26 vs 54 grams, p=0.006 T-test). Significant differences were observed in mean lymph node yields (p=0.045, T-test) and mean weights of axillary clearance (p=0.048) between surgical teams. Other factors (stage, grade, ER, PR, Her2, histological type, neoadjuvant therapy, type of excision, extracapsular spread and size of metastases) did not associate with lymph node yield. Significantly lower lymph node yields were also seen when cut-up was performed by registrars (compared to ST1s and consultants, p<0.05). Conclusions: The weight of the axillary clearance, determined, in part, by surgical techniques, is a significant factor in determining lymph node yields

LECTURE THEATRE Ⓐ

17.30–18.30 PUBLIC LECTURE

Chair: Prof S Fleming, University of Dundee

Brain Tissue Donation – a Unique Gift Essential for Neuroscience Research?

Prof J Ironside, CBE, National CJD Surveillance Unit, Western General Hospital, Edinburgh

PARLIAMENT HALL, UNIVERSITY OF ST ANDREWS

18.30–20.00 Welcome Reception

08.00 **FOYER**
REGISTRATION AND COFFEE

COMPUTER ROOM – GROUND FLOOR *(for Virtual Slides)*
ROOM 235 – BETWEEN LECTURE THEATRES Ⓐ AND Ⓑ *(for Microscope Slides)*
09.00–17.30 **SLIDE SEMINAR CASE COMPETITION VIEWING: *Head/Neck/Oral Pathology***

LECTURE THEATRE Ⓐ
09.00–12.00 **ORAL COMMUNICATIONS**
Chair: Prof CS Herrington, University of St Andrews
Dr JS Reis-Filho, Institute of Cancer Research, London
Categories: Breast; Gynaecological; Cellular/Molecular; Experimental Tumour Pathology

09.00–09.15 [**O17**] ***Epithelial Mesenchymal Transition in Invasive Breast Carcinoma: Molecular Pathways and Relation to Molecular Subtypes***

Ⓟ Aleskandarany, M[1]; Green, A[1]; Rakha, E[2]; Powe, D[1]; Ellis, I[1]

[1]School of Molecular Medical Sciences, Nottingham, United Kingdom; [2]Department of Pathology Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom

Epithelial Mesenchymal Transition (EMT), as defined by loss of epithelial characteristics and gain of a mesenchymal-like phenotype, has been reported in-vitro with much emphasis. However, in breast cancer (BC) there is a debate regarding the presence and occurrence of events defining EMT in-vivo. This study aims to explore the EMT portraits of BC with relevance to different molecular pathways and molecular subtypes. Hierarchical and k-means clustering analysis was performed on a well-defined cohort of invasive non-lobular BC (n=431), prepared as tissue microarray and a large panel of biomarkers including cadherins (cad), TGFβ1, PIK3CA, pAkt, cytokeratins, Erb-family members and hormone receptors. Within the resulting molecular subtypes (Luminal1&2, HER2+, and basal-like (BLBC)), there was differential expression of EMT markers. BLBC tended to express lower E-cad, higher P-cad, SMA and PIK3CA, relative to HER2+ BC that expressed highest levels of N-cad, TGF<β>1 and PIK3CA. Within the subdivisions of luminal tumours, expression of N-cad, TGFβ1, pAkt and PIK3CA expression levels differed considerably. Interestingly, N-cad contributed to cluster separation more than E-cad (F=13.14 & 1.68, respectively). Moreover, the E-cad/N-cad switch occurred more frequently in BLBC and HER2+ than in luminal tumours. Significant differences were observed between the four clusters for BCSS and DFS (log rank=33.27&21.03,respectively,p <0.001). Conclusion, both BLBC and HER2+ BC preferentially displayed EMT/cadherin switch than luminal BC that could explain the indigenous tendency of these subtypes for progression. In addition, EMT/cadherin switch programs in BC appear to occur in synergy with TGFβ1 and PIK3/Akt pathways activation. These data explain, at translational level, the molecular heterogeneity and varied clinical behaviour of BC molecular subtypes and could help developing targeted therapies against EMT- associated pathways.

The final cluster centres and mean H-scores for each variable				
	LUM1	LUM2	BLBC	HER2
A: Markers of EMT/Cad Switch				
1- N-Cadherin	93	145	121	170
2- E-Cadherin	123	131	109	126
3- P-Cadherin	41	42	129	91
4- Smooth muscle actin	2	7	17	9
B: TGFBeta1	81	143	116	143
C: PIK3/Akt				
1- pAKT	128	176	80	134
2- PIK3CA	78	195	208	226
D: Erb family/Receptor tyrosine kinases				
1- c-erb-B2	18	16	6	199
2- EGFR	2	9	26	17
3- c-erb-B3	99	198	135	195
4- c-erb-B4	50	172	95	169
E: Hormone Receptors				
1- ER	142	152	24	25
2- PR	166	140	3	15
F: Cytokeratins				
1- CK5/6	2	1	40	8
2- CK14	2	2	24	6
3- CK18	211	240	50	220
4- CK19	200	223	108	192

09.15–09.30 [**O18**] ***PARP1 Expression in Hormone Estrogen Receptor Negative Breast Cancer: Preferential Expression in Basal-Like and HER2-Positive Tumours***

Ⓟ Benhasouna, A; Green, A; Powe, D; Aleskandarany, M; Rakha, E; Ellis, I

University of Nottingham, United Kingdom

Nuclear poly (ADP-ribose) polymerases (PARPs) are a family of global monitor of chromatin structure and DNA damage repair. Chemical inhibitors of PARP have been explored as anticancer therapeutic agents in different solid cancers including breast cancer (BC) and in particular the basal-like class. However, the relative expression levels of PARP1 protein in estrogen receptor negative (ER-ve) BC remain unclear. Therefore the aim of this study was to investigate PARP1 protein expression in ER-ve BC with relevance to molecular subtypes and disease outcome. Methods: PARP1 protein expression was assessed, using immunohistochemistry, in a well-characterised series of ER-ve primary operable invasive BC cases (n=251) with long term clinical follow-up. Results were correlated with molecular and clinicopathological parameters and patients' outcome. Results: PARP1 nuclear expression was classified as high or low using a cut-off of 80 H-score, determined using X-Tile bio-informatics software. 135 (53.8%) of the informative cases were classified showing high expression. Significant positive correlation was found between PARP1 expression and BRCA1 expression (p=0.002) but no association was found with p53. High PARP1 expression was observed in both basal-like (HER2- and CK5/6 and or EGFR+) (60%) and HER2+ (64%) compared with triple negative (TN) non-basal BC classes (45%). PARP1 expression was significantly associated with BC specific survival (p=0.01). When analysis was assessed by the molecular subtype, the association of PARP1expression with improved survival was restricted to TN tumours. Conclusion: We have observed a positive correlation between PARP1 protein expression and BRCA1 expression. Although high PARP1 expression is seen in ER-/HER+ and HER- tumours, its association with survival was only found in the ER-/HER2- subtype. Thus, its targeted inhibition may particularly benefit patients with TN BC.

09.30–09.45 [**O19**] ***Male Versus Female Breast Cancer: Study of 523 Matched Cases Reveals Differences Behind Similarity***

Ⓟ Speirs, V[1]; Shabban, A[1]; Pollock, S[1]; Hanby, A[1]; Ball, G[2]; Male Breast Cancer Consortium, I.N.T.[3]

[1]Leeds Institute of Molecular Medicine, United Kingdom; [2]Nottingham Trent University, United Kingdom; [3]c/o Leeds Institute of Molecular Medicine, United Kingdom

Retrospective studies on male breast cancer (BC) have suffered from small numbers of cases available from any one centre. Using a co-ordinated multi-centre approach, we present the first large scale study to address the relevance of hormone receptor expression in male and female BC using immunohistochemistry combined with bioinformatics. TMAs were constructed from FFPE blocks from 260 male BCs and 263 female BCs and immunostained for ERα, ERβ1,-2, -5, totPR, PRA, PRB and AR and typed using CK5/6, 14, 18 and 19. Hierarchical clustering (HC) and principal component analysis (PCA) were conducted to determine the differential nature of influences and interactions between male and female BCs. Luminal A subgroup (ERα+ and/or PR+, HER2-) was the most common phenotype in both sexes. Luminal B (ERα+ and/or PR+, HER2+) was not seen in males, while basal-like tumours (ERα-, PR-, HER2-, CK5/6+) were infrequent in both. HC revealed common clusters between male and female BC including total PR-PRA-PRB and ERβ1/2 clusters. Strikingly, ERα occurred on distinct clusters between males and females. AR, ERβ1, -2 and -5 all existed on the same cluster but with a different sub structure particularly around the positioning of AR. ERα associated with this cluster in the male but not the female group. PCA confirmed that in both groups strong influences came from PR-PRA-PRB. In males strong influences additionally came from AR and ERβ1,-2,-5 whereas in females this came from ERα alone. In the largest comparison of male and female BC ever conducted, our data demonstrate that breast cancer is biologically different in males and females which could have implications for management. As male BC is rising, further understanding its biology is critical.

09.45–10.00 [**O20**] ***AGTR1 (Angiotensin II Receptor, Type 1) Expression and Its Prognostic Implications in the ER-Positive Luminal-Like Breast Cancer***

Ⓟ Habashy, H[1]; Powe, D[2]; Ball, G[3]; Green, A[1]; Rakha, E[2]; Ellis, I[1]

[1]Department of Pathology, School of Molecular Medical Sciences, University of Nottingham, United Kingdom; [2]Department of Cellular Pathology, Nottingham University Hospitals NHS Trust, United Kingdom; [3]School of Science and Technology, Nottingham Trent University, United Kingdom

Global gene expression and protein profiling studies suggest that oestrogen receptor (ER) positive breast contains distinct subgroups differing according to biology and clinical outcome. It has been reported that angiotensin II can mediate the growth of breast cancer via angiotensin II receptor, type 1 (AGTR1). Recently, it has been shown that AGTR1 overexpression defines a subset of ER+ breast cancer that can benefit from AGTR1 antagonists. In this study, Illumina gene expression data of 128 invasive breast carcinomas (BC) containing 47,293 transcripts were analysed by Artificial Neural Network (ANN) bioinformatics to identify biomarkers that best characterise the Luminal BC. ANN analysis identified AGTR1 as one of the best-ranked genes to differentiate between ER+ luminal (defined as ER+ and HER2/CK5/6/14 negative) and ER negative non-luminal cancers. Protein expression of AGTR1 was therefore assessed in a large series of consecutive patients (1902 patients) with long term follow up using immunohistochemistry and tissue microarray to evaluate the biological and prognostic significance of it expression in BC. AGTR1 showed an association with younger age (p=0.03) and development of distant metastasis. In the ER+ tumours, AGTR1 expression was correlated with higher mitotic counts (p=0.029) and MIB1 index

(0.042). Patients with AGTR1 expression showed shorter breast cancer specific survival (BCSS) (p=0.002) and distant metastasis free interval (DMFI) (p=0.039). Multivariate analysis showed that AGTR1 is an independent predictor of BCSS (HR=1.636, p=0.020). In conclusion, AGTR1 expression in the luminal phenotype characterises aggressive subgroup with high proliferation, shorter BCSS and shorter DMFI. Our study supports that blocking this receptor could represent an important therapeutic target in luminal-like BC.

10.00–10.15 [**O21**] ***HPV16 Episome-Associated Cervical Neoplastic Progression In Vitro Demonstrates Fundamental Similarities to Integrant-Associated Carcinogenesis***

Ⓟ Pett, M; Gray, E; Ward, D; Scarpini, C; Coleman, N

MRC Cancer Cell Unit, Cambridge, United Kingdom

An important event in the development of cervical squamous cell carcinoma (SCC) is deregulated expression of high-risk human papillomavirus (HR-HPV) oncogenes, most commonly related to viral integration into host DNA. Mechanisms of development of the ~15% of SCCs that contain extra-chromosomal (episomal) HR-HPV are poorly understood, due to limited longitudinal data. We therefore exploited the W12 model to study mechanisms of cervical carcinogenesis associated with episomal HPV16. In vitro progression of W12 normally occurs through selection of cells containing integrated HPV16. However, in one long-term culture, keratinocytes developed a selective growth advantage and invasive phenotype, while retaining HPV16 episomes at increased copy number in the absence of transcriptionally active integrants. Longitudinal investigations revealed similarities between the episome- and integrant-associated routes of neoplastic progression. Most notable were dynamic changes in viral early gene expression in episome-retaining cells, consistent with continually changing selective pressures. An early increase in viral transcription preceded elevated episome copy number and was followed by a reduction to near baseline after the development of invasiveness. Episomal transcriptional deregulation did not require selection of a specific sequence variant of the HPV16 upstream regulatory region, although increased levels of acetylated histone H4 around the late promoter implicated a role for altered chromatin structure. Interestingly, invasive episome-retaining cells demonstrated high levels of HPV16-E2/E6 proteins (despite decreased transcript levels) and reduced expression of interferon-stimulated genes, adaptations that support viral persistence and cell survival. Our findings suggest a unified working model for events important in cervical neoplastic progression, regardless of HR-HPV physical state.

10.15–10.30 [**O22**] ***Modelling Human Breast Cancer by Transforming Breast Cells with Polycomb Proteins***

Ⓟ Reynolds, P; Fedele, V

University of St Andrews, United Kingdom

The aim of this study was to test whether ectopic expression of polycomb proteins quantitatively transforms human breast epithelial cells and g enerates clinically relevant models of human breast cancer. Polycomb proteins are transcriptional repressors that remodel chromatin and have roles in development and differentiation. However, recent evidence has demonstrated a crucial role for these proteins in the control of cellular lifespan, cellular transformation and metastatic potential. We used lentiviral-mediated ectopic expression of selected polycomb proteins, together with H-Ras/ hTERT to assess whether human primary epithelial cells are transformed to malignancy under these conditions. We compared the BMI1 oncogene with the related polycomb protein CBX8 in this setting. We found that CBX8 expressing cells shared both similarities and differences to those expressing BMI1 in this model system. This work has implications for understanding the fundamental basis of breast cancer.

10.30–11.00 **Coffee [FOYER]**

11.00–11.15 [**O23**] ***Tumour Infiltration by Myeloid Derived Suppressor Cells Post Treatment with Chemotherapy May Become Incorporated into the Tumour Endothelium***

Ⓟ Reed, H[1]; Melis, M[2]; Byers, R[2]; Illidge, T[1]

[1]Paterson Institute for Cancer Research, Manchester, United Kingdom; [2]University of Manchester, United Kingdom

The presence of Myeloid Derived Suppressor cells (MDSCs) in tumours is associated with increased tumour growth, tumour regrowth after anti-cancer treatment and host immune T-cell suppression against tumour. The role of MDSCs in promoting tumour growth via tumour angiogenesis and vasculogenesis is less well defined. We aimed to 1) investigate a subpopulation of MDSCs defined by the cell surface markers CD11b and Gr1 in tumours after cytotoxic treatment and 2) to investigate the potential role of these CD11b+Gr1+ cells in the formation of tumour vasculature. This preliminary study was performed to examine the potential role of MDSCs in tumour regrowth after treatment with chemotherapy and radiotherapy. Mice were injected subcutaneously with B16 (melanoma) tumour cells and treated 7 days after tumour inoculation with either 150ug Doxorubicin or 15Gy local external beam radiation. Tumours were collected 1 to 7 days after treatment and analysed using dual quantum dot immunohistochemistry, which enabled accurate quantification and co-localisation of the biomarkers measured: CD11b, Gr1, F4/80 and CD31. Our results demonstrated a significant increase in infiltration of CD11b+Gr1+ cells into the tumour one day after treatment ($p<0.01$). Using the endothelial marker CD31, we determined that there was a highly significant increase in the co-localisation of CD11b+ and CD31+ cells after chemotherapy (80%) than in the non-treatment control group (37%) ($p<0.001$). Similar findings post radiation therapy were also seen. Our findings suggest that the increased co-localisation of CD11b+ cells within the CD31+ population represents an immature myeloid cell population differentiating into endothelial cells. This data supports the hypothesis that

MDSCs can be directly incorporated into the tumour endothelium and may play an important role in tumour angiogenesis.

11.15–11.30 [**O24**] ***Investigating Clonal Dynamics in Barrett's Associated tumorigenesis Using Spatial Maps of Genetic Heterogeneity***

Ⓟ Graham, T[1]; Khan, S[1]; Oukrif, D[2]; Green, A[3]; Jankowski, J[3]; Barr, H[4]; Novelli, M[2]; Shepherd, N[4]; Leedham, S[5]; McDonald, S[3]; Wright, N[3]

[1]Cancer Research UK, London, United Kingdom; [2]University College London, United Kingdom; [3]Barts and the London School of Medicine and Dentistry, United Kingdom; [4]Gloucestershire Royal Hospital, Gloucester, United Kingdom; [5]Oxford University, United Kingdom

Purpose of study To fully characterise clonal structure and dynamics within Barrett's tissues. Genetic heterogeneity within Barrett's mucosa is significantly associated with the development of oesophageal adenocarcinoma (OA), though why this association exists is unclear. In order to fully characterise genetic diversity in within Barrett's tissue, we have undertaken a detailed clonal-ordering analysis to produce phylogenetic trees and spatial maps that illustrate the development and spread of genetically distinct clones. Methods Endoscopic mucosal resection specimens (EMRs) were serially sectioned. Crypts were classified according to histological grade. Aneuploidy was assessed using image cytometry. More than 50 individual crypts were then laser-capture microdissected from each EMR. The genetic mutation burden of individual crypts was assessed (K-ras, TP53 and P16 point mutations and microsatellite loss of heterozygosity (LOH) data for up to16 loci on chromosomes 3p (FHIT), 5q (APC), 9p (P16), 17p (TP53), 17q and 18q (SMAD4)). These data were used to construct a genetic and histological map of each EMR. Summary of Results EMRs were rarely genetically homogeneous. Typically, dysplastic crypts within individual lesions were clonal for a founding TP53 or P16 point-mutated clone, whereas the adjacent metaplastic (non-dysplastic) crypts analysed did not contain the point mutation. Mosaic subclones with distinct patterns of LOH (often 18q and/or 3p) were often present. Conclusions Clonal expansion of mutated cells is frequently restricted to small numbers of crypts. Interactions between genetically distinct clones appear to be predominantly competitive: a crypt acquiring an additional pro-tumorigenic mutation outcompetes the original crypts. Mutations of the tumour suppressor genes TP53 and P16 are not found in non-dysplastic tissue.

11.30–11.45 [**O25**] ***Targeting Cdc7 Kinase Shields Normal Cycling Cells From Taxane Cytotoxicity and Increases Cancer Cell Killing***

Ⓟ Williams, G; Kingsbury, S; Chen, L; Wollenschlaeger, A; Sainsbury, R; Stoeber, K

University College London, United Kingdom

Cdc7 kinase is essential for initiation of DNA synthesis at chromosomal replication origins. Loss of Cdc7 kinase activity through RNA interference or treatment with small molecule inhibitors is known to cause cancer cell-specific killing. This differential response between normal and transformed cells has been linked to a p53-dependent checkpoint that arrests cell cycle progression at the G1-S boundary when Cdc7 activity is low. Many cancer cell types have an impairment of this checkpoint due to p53 mutations and therefore enter S phase with an insufficient number of replication forks resulting in apoptotic cell death.

Here we demonstrate that primary cells arrested by the Cdc7-inhibition checkpoint are shielded from the cytotoxic effects of the potent anti-mitotic agent paclitaxel, whereas combined CDC7-siRNA and paclitaxel treatment results in increased cancer cell-specific killing.

Thus sequential treatment with Cdc7 inhibitors and broadly used anti-mitotic agents may increase cancer cell killing while preventing associated toxicity in normal proliferating tissues, thereby overcoming the Achilles heel of cyclotherapy.

11.45–12.00 [**O26**] ***p53 Has an Unexpected Role in the Regulation of the Cytokinesis Associated Protein Anillin***

Ⓟ Hall, P; Ma, J; Borthwick, K; Russell, S

Queen's University, Belfast, United Kingdom

The expression of the anillin is regulated in a cell cycle dependent manner in terms of its mRNA and protein level and its spatial localization. In interphase anillin protein is found in the nucleus, while levels increase in late metaphase when anillin localizes to the developing cleavage furrow. This 1025 amino acid protein acts as an adapter with the potential to bind membranes and septins, actin, microtubules, myosin II, and signaling molecules such as rho. We have described anillin over-expression in diverse forms of neoplasia with levels that increase with advancing clinical stage (*Clin Cancer Res* 2005;11:6780-6). The mechanism of this deregulation is unclear. We hypothesized that anillin is a p53 repressed gene and that the commonly observed loss of function of p53 in tumours might explain this phenotype with loss of p53 function leading to loss of anillin repression. Bioinformatic analysis of the anillin promoter revealed putative p53 responsive elements. We have shown that two of these sites have the ability to bind p53 protein by chromatin immunoprecipitation assay. Furthermore DNA sequences encompassing these sites can regulate luciferase transgenes in a p53 dependent manner while mutant p53 fails to activate these constructs. Direct analysis of anillin protein shows that it is positively regulated by DNA damage in p53 wild type but not in isogenic p53 null cells. These data indicate that anillin is positively (rather than negatively) regulated by p53 and hence the common overexpression of anillin in cancer is not a p53 mediated phenomenon. However our data sheds an entirely new perspective on p53 function where it has roles in the biologically crucial process of cytokinesis, opening a wholly new area for study.

LECTURE THEATRE G

09.00–12.00 **TRAINEES' SYMPOSIUM:** ***Molecular Pathology: What is it and what can it tell us?***

Chair: Dr I Proctor, University College London

09.00–09.50 [**S6**] ***Molecular Biology for Beginners***

Dr A Jubb, University of Oxford

This presentation will review the basics of molecular biology and how these techniques are utilized in academic pathology. The aim is to provide a fundamental understanding for the uninitiated to better appreciate the presentations that follow.

09.50–10.40 [**S20**] ***Molecular Frontiers of (Breast) Pathology***

Dr JS Reis-Filho, Institute of Cancer Research, London

Institute of Cancer Research, Breakthrough Breast Cancer Centre, United Kingdom

Breast cancer is a heterogeneous disease, comprising numerous distinct entities that not only have different biological features but also clinical behaviour. There are several lines of evidence to suggest that breast cancer is, in fact, a collection of different diseases with different risk factors, clinical presentations, pathological features, response to therapy and outcomes, which affect the same anatomical organ and originate in the same anatomical structure (i.e. the terminal duct-lobular unit). Although histopathologists have been aware of the diversity of breast cancer and have endeavoured to devise approaches to classify the disease into meaningful groups, the concepts of breast cancer diversity and heterogeneity have only been brought to the forefront of breast cancer research after the publication of high throughput microarray-based class discovery studies that unravelled the existence of multiple molecular subtypes and class prediction studies that identified a number of prognostic and predictive 'gene signatures'. Initially perceived as a replacement for the clinicopathological parameters employed to determine the therapy of breast cancer patients, the prognostic and predictive power of microarrays has been shown to be complementary to rather than a replacement for traditional clinicopathological parameters. Despite the limited impact so far on prognostication and prediction, integrative high throughput analyses of primary breast cancers and breast cancer models have led to the identification of novel potential therapeutic targets, which are currently being tested in prospective clinical trials (e.g. FGFR1). In this talk, we will discuss the contribution of microarray-based studies of breast cancer and new technologies (e.g. massively parallel sequencing), which will certainly impact on breast cancer classification, prognostication and prediction.

10.40–11.10 **Coffee [FOYER]**

11.10–12.00 [**S7**] ***Haematopathology in the Molecular Era***

Dr EJ Soilleux, John Radcliffe Hospital/University of Oxford

Molecular tests are now used routinely in haematopathology, complementing morphological analysis and immunohistochemistry. Economic considerations mean that their use is restricted to the proportion of cases in which they make an important distinction between differential diagnoses, which require different treatments. During lymphocyte development, a complex process of receptor rearrangement occurs, because germline encoding of the huge diversity of antigen receptors required for functional immunity would be impossible without a very large expansion of the genome. As a consequence of this process, each T or B cell receptor essentially has a unique DNA sequence. Proliferations of lymphocytes, all of which contain the same sequence, have arisen from a single progenitor lymphocyte and are known as a clone. The majority of such clones are neoplastic and can be detected by PCR based studies. A second consequence of the receptor rearrangement process is the acquisition of translocations involving the lymphocyte receptor loci and various oncogenes. Characteristic translocations are seen in particular types of lymphoma and these can be detected by fluorescent in situ hybridisation (FISH). This session will focus on the biological basis and principles of the major molecular techniques used in haematopathology and their use in clinical practice, with examples to illustrate the importance of clinicopathological correlation.

LECTURE THEATRE A

12.00–13.00 **KEYNOTE LECTURE**

Chair: Prof FA Carey, Ninewells Hospital and Medical School, University of Dundee

12.00–13.00 [**S8**] ***Colorectal Cancer Screening – Pilot's Programme***

Prof R Steele, Ninewells Hospital & Medical School, University of Dundee

Evidence from population based randomised controlled trials indicates that introduction of a Colorectal Cancer Screening Programme based on biennial guaiac faecal occult blood testing has the potential to reduce disease specific mortality by around 20%. As a result of this evidence, on the advice of the National Screening, the UK Health Departments commissioned a pilot of colorectal cancer screening which was carried out in Coventry at Warwickshire in England and in Grampian, Tayside and Fife in Scotland. In Scotland the pilot ran from March 2000 to July 2007 and consisted of three rounds of screening. The results of this pilot indicated that it was feasible to introduce a screening programme based with faecal occult blood testing and that the expected mortality

reduction was achievable. As a result roll out of screening was commenced in July 2009 and was completed by December 2010 so that all individuals aged between 50 and 75 in Scotland are now being offered biennial faecal occult blood test screening. During the pilot and initial roll out there has been an active programme of research in three main areas. 1. Analysing the data from the pilot to better understand the screening process. 2. Developing and trialling interventions to increase uptake. 3. Attempts to improve the sensitivity and specificity of the test. In this lecture in addition to an account of the piloting and roll out processes three examples of projects in the three main research work streams will be described.

FOYER

13.00–14.00 LUNCH AND TRADE EXHIBITION

LECTURE THEATRE C

14.00–15.00 RENAL PATHOLOGY EQA MEETING

FOYER

14.00–15.00 POSTER VIEWING AND CHAIRMAN'S ROUNDS

CATEGORY	POSTER NUMBERS
Cellular/Molecular	P41–P46[1]
Education and Audit	P47–P60[2]
Experimental Tumour Pathology	P61[1]
Neonatal/Paediatric	P62–P82[3]

Chair: [1]Dr RJ Byers, Manchester
[2]Dr PJ Gallagher, Southampton
[3]*(to be confirmed)*

LECTURE THEATRE A

15.00–17.30 ORAL COMMUNICATIONS

Chair: Prof A Riches, University of St Andrews
Dr RJ Byers, University of Manchester
Categories: Cellular/Molecular; Gastrointestinal

15.00–15.15 [**O27**] ***Multidimensional Analysis of Cell Cycle Regulators Identifies Prognostically Distinct Clusters in Breast Cancer***

Ⓟ Elsheikh, S[1]; Green, A[2]; Soria, D[2]; Ellis, I[3]

[1]Royal Infirmary of Edinburgh, United Kingdom; [2]Nottingham University, United Kingdom; [3]Nottingham University Hospital, United Kingdom

Introduction: Cell cycle regulators (CCR) work as a web of complex regulatory positive and negative feedback mechanisms and interconnections that regulate cell cycle progression.
In breast cancer (BC), although previous studies identified aberrant expression of CCR, there is still a need to explore the complexity of these regulators through a multidimensional analysis in order to identify different clusters of CCR and assess the ability to classify BC into clinically relevant groups.
Methods: Expression of CCR proteins (p16, p21, p27, p53, bcl-2 and c-myc) were determined on 600 cases of BC by immunohistochemistry (IHC) and semi-quantitatively assessed using H-score. Mathematical clustering was performed using K-means and PAM methods. Decision tree and WEKA software were used to propose a rule that defined clusters based on definite value.
Results: K-means and PAM algorithms assigned patients (n=520) into three clusters. Cluster 3 (high p16, high p53, low p27) was associated with adverse prognostic parameters (95% grade 3, 75% tumour size >2cm, 71% basal phenotype). Moreover, patients assigned to Cluster 3 had the shortest 10-year breast cancer specific survival, disease free survival and metastasis free survival ($p<0.001$, $p=0.001$, $p<0.001$ respectively). Multivariate analysis revealed that CCR clusters had independent prognostic power in comparison to the well-established prognostic parameters in BC.
Conclusion: CCR clusters can classify BC into prognostically distinct groups. Validation of these clusters in larger number of patients, with uniform IHC staining and scoring is mandatory before adoption into clinical practice.

15.15–15.30 [**O28**] ***Inter-Cellular Activation of DNA Damage Signalling Following Radiation Treatment***

Ⓟ Burdak-Rothkamm, S[1]; Rothkamm, K[2]; Prise, K[1]

[1]Queen's University Belfast, United Kingdom; [2]Health Protection Agency, Didcot, United Kingdom

DNA damage response pathways are a common target for mutations in tumour cells. DNA damage induced during radiotherapy activates cell cycle checkpoint and DNA repair proteins. Evidence of DNA damage has been observed

both in directly targeted and non-irradiated bystander cells which communicate with irradiated cells, e.g. via cytokine signalling.
Using immunocytochemistry-based imaging techniques we have investigated the activation and recruitment of DNA damage response proteins to sites of radiation-induced DNA damage. Significant differences in the response of targeted and bystander cells were observed. Bystander cells showed ATR dependent formation of gamma-H2AX foci in S-phase cells with subsequent activation of ATM and specific DNA repair proteins related to replication associated DNA damage repair and homologous recombination. In contrast, for targeted cells gamma-H2AX foci formation was mediated by ATM and DNA-PK independent of ATR. Inhibitors of ATR and ATM activation differentially modulated radiosensitivity in targeted and bystander cells with a decrease in clonogenic cell survival in directly targeted cells but increased resistance in bystander cells.
Our results stress the importance of a distinction between cellular radiation responses of directly targeted cells and bystander cells and provide a rationale for differential modulation of these components by molecular targeted drugs. Furthermore, screening of individual tumours for mutations in DNA repair pathways will assist the prediction of the tumour response to radiotherapy, taking into account effects on both targeted and bystander cells.
This work is supported by a Breast Cancer Campaign project grant.

15.30–15.45 [**O29**] ***Amplification of* MDM2 *and* MDM4 *in Osteosarcoma***

Ⓟ Duhamel, L[1]; Ye, H[2]; Halai, D[2]; Idowu, B[2]; Presneau, N[1]; Flanagan, A[1]

[1]Cancer Institute, University College London, United Kingdom; [2]Department of Histopathology, Royal National Orthopaedic Hospital, Stanmore, United Kingdom

Amplification of *Mouse Double Minute 2* (MDM2), which inhibits p53, has been reported in parosteal osteosarcomas (OS) making this subtype a candidate for treatment with Nutlin-3A, an inhibitor targeting MDM2 that induces apoptosis in OS cell lines in vitro and in vivo. However, when overexpressed, *Mouse Double Minute 4* (MDM4) has been reported to counteract this effect. We therefore investigated the incidence of *MDM2* and *MDM4* amplification in OS subtypes.
Interphase fluorescent *in situ* hybridization (FISH) was performed on tissue microarrays using probes for *MDM2* and *MDM4* and their respective centromeres (*Zytolight* SPEC MDM2/CEN12 (Z/2013) and MDM4/CEN1 (Z/2080) Dual colour Probe kit, Zytovision, Germany).
We confirmed that *MDM2* amplification was a common event in parosteal OS, with 19/24 (79%) informative cases showing amplification (>30% of cells with >10 MDM2 copies and 2 centromeres). Of note is that 14 contained a coexistent high grade component, compared to 1/5 cases without amplification. None of the parosteal OS showed *MDM4* amplification.
MDM2 amplification was a rare event, 6/155 (4%), in the high grade OS (3 osteoblastic, 2 mixed fibroblastic/osteoblastic, 1 undifferentiated). MDM4 amplification only occurred in 2 cases both of which also showed low numbers of cells (<30%) with MDM2 copy number gain.
FISH analysis also revealed that aneusomy of Chr 12 and Chr 1 was present in many high grade OS (91/155 and 117/169 respectively).
To conclude, the data imply that OS with *MDM2* amplification are potentially targetable with Nutlin-3A providing coexistent p53 mutations and *MDM4* amplification are excluded. However, studies are warranted to determine the effect of Chr 12 and Chr 1 aneusomy on the responsiveness to Nutlin-3A.

15.45–16.00 [**O30**] ***Network Analysis of the Cancer Trancriptome.***

Ⓟ Doig, T[1]; Gregory, C[1]; Goodlad, J[2]; Freeman, T[3]

[1]Centre for Inflammation Research, Edinburgh, United Kingdom; [2]Lothian University NHS Trust, Edinburgh, United Kingdom; [3]The Roslin Institute, Edinburgh, United Kingdom

Analysis of gene expression data from human tumours is complicated by the immense heterogeneity of the tumours. We describe a novel network-based approach to analysis of the human cancer transcriptome, which harnesses this heterogeneity to generate signatures of co-expressed genes, hypothesising that the relative contribution of any single mRNA to the total mRNA pool of an individual tumour will reflect the degree of activity or abundance of the pathway in which that gene is involved, and thus identifing clusters of genes representing individual cell types or processes within the tumour. The network analysis tool Biolayout*Express*^{3D} was used to analyse 7 large publically available gene expression datasets representing > 1100 individual tumours. A transcript-to-transcript Pearson correlation matrix was constructed for each of the seven data sets and an undirected network graph created where each node represented a transcript and edges between nodes represented pairwise correlation coefficients for each of the transcripts present on the array. A further graph was created using mean probe-probe correlations from 6 datasets to identify signatures conserved across datasets and validated using an independent dataset. Signatures were identified which were specific to tissue and tumour types as well as signatures common to all tumours. The signatures conserved across 7 tumour datasets included those associated with the microenvironment, including signatures enriched in genes expressed by macrophages, T cells, endothelial cells, and the extracellular matrix. Signatures representing elements of the cell cycle, interferon response and structures such as the ribosome were also present across datasets. We present a novel view of the cancer transcriptome which provides an insight into the intact microenvironment of the tumour across a genetically heterogeneous human population.

16.00–16.30 **Coffee [FOYER]**

16.30–16.45 [**O31**] ***Cten (C-terminal Tensin-Like) Connects Kras to Integrin-linked Kinase (ILK) and is Associated with Metastasis in Colorectal Cancer.***

Ⓟ AlGhamdi, S; Albasri, S; Lobo, D; Zaitoun, A; Durrant, L; Seth, R; Kindle, K; Fadhil, W; Ibrahim, S; Nateri, A; Ilyas, M

University of Nottingham, United Kingdom

C-terminal tensin-like (Cten, TNS4) is a member of the tensin gene family which we have previously shown results in enhanced cell motility in colorectal cancer (CRC) cell lines. Since Tensin family proteins localise to the cytoplasmic tails of integrins we hypothesised that Cten may act through Integrin-linked kinase (ILK). Knockdown and forced expression of Cten in the CRC cell lines SW620 and HCT116 (respectively showing high and low Cten expression) showed that Cten up-regulates ILK. Furthermore, concomitant knockdown of ILK and forced expression of Cten in HCT116 resulted in loss of the motility inducing effects of forced Cten expression. Since the EGFR has been shown to regulate Cten, we tested whether this may be mediated through Kras signalling. Knockdown of mutant Kras in SW620 resulted in down-regulation of Cten and inhibition of cell motility. Forced expression of Cten with Kras knockdown however restored cell motility to normal. Finally, we tested a series of 476 CRCs for expression of Cten by immunostaining. High Cten expression was significantly associated with advanced Tstage, vascular invasion and distant metastasis. Univariate analysis showed that high expression was associated with poor prognosis ($p<0.001$) and a trend was found on multivariate analysis ($p=0.07$). We propose that there is a Kras – Cten – ILK axis regulating cell motility and tumour metastasis.

16.45–17.00 [**O32**] ***Activating K-Ras Mutations Outwith "Hotspot" Codons in Sporadic Colorectal Tumours– Implications for Personalised Cancer Medicine***

Ⓟ Smith, G; Bounds, R; Wolf, H; Pratt, N; Carey, F; Steele, R; Wolf, C

University of Dundee, United Kingdom

Response to cetuximab (Erbitux®) and related EGFR-targeted therapies in colorectal cancer patients with metastatic disease has been convincingly associated with K-Ras mutation status, where response is restricted to patients with "wild-type" K-Ras. Current mandatory mutation testing for patient selection is limited to the K-Ras "hotspot" codons 12 and 13. We have investigated whether K-Ras mutations are present outwith previously described hotspot codons, and have used a variety of experimental approaches to compare and contrast the phenotypes associated with individual K-Ras mutations. A cohort of unselected, sporadic colorectal tumours (n=106) was screened for additional K-Ras mutations, and phenotypes compared in transformation and Ras GTPase activating assays. Gene and pathway changes induced by individual K-Ras mutations were identified by microarray analysis. Taqman-based gene copy number and FISH analyses were used to investigate a novel K-Ras gene amplification event, the presence of which was initially suggested by CGH analysis. Four additional K-Ras mutations ($Leu_{19}Phe$ (1/106 tumours), $Lys_{117}Asn$ (1/106), $Ala_{146}Thr$ (7/106) and $Arg_{164}Gln$ (1/106)) were identified. $Lys_{117}Asn$ and $Ala_{146}Thr$ had phenotypes similar to the hotspot mutations, while $Leu_{19}Phe$ had an attenuated phenotype and the $Arg_{164}Gln$ mutation was phenotypically equivalent to wild-type K-Ras. We additionally confirmed the presence of a novel K-Ras gene amplification event, present in approximately 2% of tumours. The identification of mutations outwith previously described hotspot codons increases the K-Ras mutation burden in colorectal tumours by one third. Future mutation screening to facilitate optimal patient selection for treatment with EGFR-targeted therapies should therefore be extended to codon 146, and should additionally consider the unique molecular signatures associated with individual K-Ras mutations.

17.00–17.15 [**O33**] ***Cell Surface Proteomics Identifies Prion Protein as a Candidate Biomarker for Colorectal Adenoma-to-Carcinoma Progression***

de Wit, M; Jimenez, C; Carvalho, B; Belien, J; Delis - van Diemen, P; Mongera, S; Piersma, S; Meijer, G; Ⓟ Fijneman, R

VU University Medical Center, Netherlands

Purpose of the study: Early diagnosis of colorectal cancer (CRC) is a realistic approach to reduce its high mortality rates. Currently available methods for early detection of CRC do not distinguish the 5% of adenomas expected to progress into CRC (high-risk adenomas) from adenomas with low risk of progression. We aimed to identify cell surface protein biomarkers that can be targeted for molecular imaging and discriminate low-risk adenomas from high-risk adenomas and CRC.

Methods: Genome-wide mRNA profiling revealed genes with increased expression in CRCs compared to adenomas. To identify the subset of genes encoding plasma membrane-bound proteins, extracellular domains of cell surface proteins of five CRC cell lines were biotinylated, isolated, and analysed by in-depth proteomics using gel electrophoresis and nanoliquid-chromatography coupled to tandem mass spectrometry. Cell surface expression was confirmed by FACS analysis and immunohistochemistry.

Summary of results: In total 2609 proteins were identified in the cell surface fractions. Of these, 31 candidate biomarkers were selected based on protein identification in at least four cell lines, a predicted (trans)membrane location, and increased mRNA expression in CRC compared to adenomas ($p<0.05$). For one candidate, the cellular prion protein PrPc, increased expression in a series of CRCs compared to adenomas was verified by immunohistochemical evaluation.

Conclusions: Our strategy successfully yielded cell surface candidate biomarkers for molecular imaging of adenoma-to-carcinoma progression, exemplified by identification of PrPc. Moreover, this study illustrates that panels of biomarkers will be required to cover detection of the full spectrum of the molecularly heterogeneous group of high-risk adenomas and CRCs.

17.15–17.30 [**O34**] ***Stem Cell Location Throughout the Human Gastrointestinal Tract as Defined by* LGR5 *mRNA* In Situ *Hybridisation***

Ⓟ McDonald, S[1]; Jeffery, R[2]; Humphries, A[2]; Leedham, S[3]; Graham, T[2]; Segditsas, S[3]; Lewis, A[3]; Nicholson, A[1]; Bamba, S[4]; Rodriguez-Justo, M[5]; Novelli, M[5]; Jankowski, J[1]; Wright, N[1]; Poulsom, R[2]

[1]Barts and the London, United Kingdom; [2]Cancer Research UK, London, United Kingdom; [3]Oxford University, United Kingdom; [4]Shiga University of Medical Science, Shiga, Japan; [5]University College London, United Kingdom

Background: Attempts to identify stem cells within the gastrointestinal tract have had limited success, as existing markers do not demonstrate multilineage differentiation. Recently, the leucine-rich-repeat-containing G-protein-coupled receptor 5 (LGR5) has been shown to reveal crypt base columnar cells capable of dividing into all lineages and are therefore stem cells. *Aims*: To provide the first localization of *LGR5* mRNA +ve cells throughout the normal and premalignant human gastrointestinal tract using mRNA *in situ* hybridisation. *Methods*: Formalin-fixed, paraffin-embedded gastrointestinal normal/metaplastic/dysplastic tissue blocks were used from patients that had undergone resection for a gastrointestinal adenocarcinoma. Localisation of *LGR5* mRNA was accomplished by *in situ* hybridisation using an ^{35}S-UTP antisense probe. The presence of hybridisable mRNA was established in serial sections using an antisense β-actin probe. *Results*: Distribution of *LGR5* mRNA +ve cells throughout the human gastrointestinal appeared to be restricted to the neck of normal gastric glands and at the base of intestinal crypts. Human intestinal metaplasia, whether Barrett's or gastric tissue, had *LGR5* mRNA +ve cells located at their crypt base, similar to the expression seen throughout the intestine. Dysplastic glands in the stomach showed expansion of *LGR5* mRNA +ve cells from the base to approximately half way up glands. In both gastric and oesophageal adenocarcinoma *LGR5* mRNA was evident at the tumour margin and within invasive component. *Conclusion*: Here we have localized *LGR5* mRNA +ve cells within the normal and premalignant gastrointestinal tract. It appears that, in normal gastrointestinal epithelium at least, *LGR5* mRNA is localised to cells concordant with established lineage tracing in the mouse although not within the stomach. The *LGR5* +ve population expand in metaplasia and adenomas and become widespread in certain tumours.

LECTURE THEATRE Ⓑ

15.00–17.00 SYMPOSIUM: *Pathology in the Developing World*

Chair: Prof FA Carey, Ninewells Hospital & Medical School, University of Dundee
Prof S Guild, University of St Andrews

15.00–15.20 [**S9**] ***The Scotland - Malawi Pathology Partnership***

Prof FA Carey, Ninewells Hospital & Medical School, University of Dundee

As part of a broader series of health links the Scottish Pathology Network (SPAN) has developed a partnership with the Department of Pathology at the College of Medicine (COM) in Blantyre, Malawi. COM is the sole provider of histopathology in Malawi (pop. 13 m) and, at present, there is only one reporting senior pathologist. The Scottish link began in 2007 and has involved supporting undergraduate teaching and diagnostic pathology. Seven participants have visited for between 3-4 weeks. Some have visited more than once. The project now wishes to link to broader UK/European initiatives in developing postgraduate training and improving service quality. The contribution of RCPath, BDIAP and the Pathological Society of Great Britain and Ireland will be key to developing a sustainable link.

15.20–15.40 [**S10**] ***Initiatives in Laboratory Medicine in Developing Countries and the Role of the RCPath***

Dr I Bates, Liverpool School of Tropical Medicine

The increasing burden of infectious diseases such as HIV, malaria and TB coupled with changing standards and regulatory requirements mean that many laboratory services in the world's poorest countries are struggling to provide even a basic service. Chronic neglect has led to crippling staff shortages, inadequate funding, poor quality reagents and unreliable equipment. This situation is changing as international policy makers and funding agencies begin to recognise the critical role that laboratory services play in underpinning all aspects of healthcare. Many laboratory clinicians and scientists from the UK have experience of working and living in developing countries and have contributed to supporting their laboratory services. These efforts have generally not been widely disseminated, and there is no mechanism for learning lessons, for synergising or for avoiding duplication. Several recent government strategies urge UK institutions to increase their inputs to healthcare in poorer countries and there are several laboratory medicine initiatives (viz: the session on Pathology in the Developing World) that are putting this into practice. The RCPath is committed to playing a pivotal role in coordinating and expanding role of UK expertise in developing laboratory medicine in low-income countries.

15.40–16.00 [**S11**] ***Undergraduate Education, the St Andrews-Malawi Link***

Prof S Guild, University of St Andrews

The University of St Andrews is collaborating with College of Medicine (COM) Blantyre to assist the College with a major review of the undergraduate medical curriculum. The changes are driven by the need to modernize the curriculum content and its delivery, and significantly increase the number of medical students in training. This is a major priority of the Malawian Government. The Bute Medical School (BMS) has recently undergone its own

major curriculum review and redesign for similar reasons and so is able to offer advice based on its own relevant experience. As a result of two joint conferences in Blantyre, the COM implemented a new, spiral curriculum in January 2009. The content is organised in integrated modules in the first 2 years in which the clinical context of basic medical science is established from the beginning. The first turn of the curriculum spiral lays the foundation for a systems-based approach for the remainder of the first two years. The content of the systems-based modules is presented in a recognized cycle of normal structure, followed by normal function, abnormal function and finally by treatment. Assessments are based on published learning outcomes linked to all learning activities. The aim is to create an integrated series of assessments that are valid, reliable and blueprinted to specific appropriate learning objectives. Years 3 to 5 in the new curriculum move to hospital-based rotations but the underlying philosophy of integration of the sciences with clinical practice is sustained. Vertical themes of 'Golden Topics' particularly relevant to health in Malawi are developed throughout the 5 year programme. The delivery of the curriculum is supported by a bespoke online curriculum management system developed in collaboration between the two schools.

16.00–16.30 **Coffee [FOYER]**

16.30–16.50 **[S12]** ***British Division of the International Academy of Pathology African Developments***

Dr AJ Howat, East Lancashire Hospitals NHS Trust, Burnley, United Kingdom

The International Academy of Pathology (IAP) is a global body of divisions dedicated to the advancement of Pathology through educational exchanges worldwide. Recently the IAP has encouraged financially healthy divisions to form relationships with those from underserved areas of the world. The British Division of the IAP (BDIAP) has been developing links in sub-Saharan Africa (SSA) especially helping the new East African Division of the IAP (EADIAP). In 2008 I attended the APECSA meeting in Mombasa, Kenya; at that time I interviewed as many local pathologists as I could, made enquiries from knowledgeable colleagues and produced a report for the BDIAP (available on www.bdiap.org). That report identified the weaknesses in many laboratories in SSA and formed the following action plan: 1. immediate practical help including shipping new and used books 2. educational help including an annual 'safari' and establishment of an annual EA-B School of Pathology 3. support FNA cytology 4. improve technical quality of the service 5. provide a second opinion service if desired 6. continue to send educational ambassadors to APECSA, SADIAP & EADIAP meetings 7. fund trainees from EA in South Africa and/or UK 8. encourage locums by UK staff in EA. All these actions have/are being achieved. In addition, the BDIAP is aiding the development of a College of Pathology in the area (COPECSA). Finally the BDIAP is working with UK partners to coordinate efforts.

16.50–17.00 **Discussion**

07.30 **FOYER**
REGISTRATION AND COFFEE

LECTURE THEATRE Ⓒ

07.45–09.00 **TRAINEES' BREAKFAST SESSION** *(Breakfast will be served)*
Chair: Dr A Jubb, University of Oxford

[**S13**] ***Ovarian Cancer Pathology***
Prof CS Herrington, University of St Andrews

Ovarian cancer pathology Recent evidence suggests that the traditional classification of primary epithelial tumours of the ovary requires some modification, with delineation of distinct categories, some of which cross-traditional morphological boundaries. High-grade serous and endometrioid tumours, and undifferentiated carcinomas, have a common association with p53 mutation and loss of BRCA1/2 function. Low-grade serous carcinomas are associated with serous borderline tumours and mutations in the BRAF/KRAS genes. Similarly, mucinous carcinomas are associated with borderline mucinous tumours and KRAS mutation. Low-grade endometrioid tumours are associated with ovarian endometriosis and mutation of the β-catenin and PTEN genes. There is also recent evidence that there are clinico-pathological differences between clear cell carcinomas that arise in association with endometriosis, and those that are associated with clear cell adenofibroma. There is evidence that high-grade serous carcinomas of the ovary can develop directly from ovarian surface epithelium or Mullerian inclusions. Careful morphological assessment of Fallopian tube specimens has led to an additional hypothesis, namely that these tumours, and other pelvic serous carcinomas, may also arise from the epithelium of the distal end of the Fallopian tube. Focal abnormalities of p53 expression have been identified in morphologically benign tubal epithelium (p53 signatures), and these abnormalities form a spectrum with tubal intraepithelial carcinomas (TICs). As many ovarian carcinomas present late, such small precursor lesions could be destroyed by tumour progression, or overlooked during histopathological assessment. This concept has significant implications for the mechanisms that underly the development of pelvic high-grade serous carcinomas and may help to identify novel approaches to the treatment of these tumours.

COMPUTER ROOM–GROUND FLOOR *(for Virtual Slides)*
ROOM 235–BETWEEN LECTURE THEATRES Ⓐ AND Ⓑ *(for Microscope Slides)*

09.00–17.30 **SLIDE SEMINAR CASE COMPETITION VIEWING: *Head/Neck/Oral Pathology***
(Note competition closes at 15.00)

LECTURE THEATRE Ⓐ

09.00–13.00 **SYMPOSIUM: *Viruses and Disease***
Chair: Prof CS Herrington, University of St Andrews

09.00–09.40 [**S14**] ***The Pathology of HIV Infection***
Prof SB Lucas, King's College London, School of Medicine

Over 30 years of study, the pathology of HIV infection has expanded as the epidemic has matured globally and anti-HIV treatment (cART) has become available. The pathogenesis, once considered to be simply the cytopathic destruction of CD4+ T-cells with ensuing disintegration of cell-mediated immunity (CMI), has become more complicated: HIV disease is now a chronic immune activation process with secondary endothelial cell damage and lympho-reticular system disorganisation. The early-described major HIV-associated pathologies (PCP, KS, tuberculosis, lymphoma etc) have not altered, but their frequencies have changed with cART, some in unpredicted ways. New entities are described, eg HHV8-associated multi-centric Castleman disease, and HIV-related haemophagocytic syndrome, which are causes of multi-organ failure and mortality in HIV. New encephalopathic syndromes are emerging in long-term treated patients, eg CD8+ T-cell enceph-alitis, and Alzheimer-like dementia. Hodgkin disease is emerging as more common and atypical than anticipated. HCV co-infection in the liver is a major morbidity in some HIV+ populations. LGV rectal infection is a major new IDB. cART (combination anti-retroviral therapy) has dramatically prolonged survival, but introduces its own toxicities, particularly in the liver (steatosis & lactic acidosis; non-cirrhotic portal hypertension), kidney (tubular injury), and possibly the heart (accelerated atheroma). The rapid improvement in CMI immune status with cART often results in immune reconstitution inflammatory syndromes (IRIS), bringing out occult infections or inducing clinical relapses. As HIV cohorts on cART live longer and age, interactions of HIV and ageing will be more common: cardiovascular disease, COPD, non-AIDS defining cancers (eg lung, anus, liver). Controversies continue over the association of HIV with pathogen-negative enteropathy, myocarditis, pulmonary hypertenion, pre-eclampsia.

09.40–10.20 [**S15**] ***Human Papillomavirus Infection and Head and Neck Cancer***
Dr E-J Speel, Maastricht University Medical Centre

Each year, ~650,000 patients worldwide are diagnosed with head and neck cancer and ~350,000 die from this disease. Besides smoking and alcohol consumption, which are causative factors for ~80% of tumours, infection with high-risk human papillomavirus (HPV) is responsible for most of the remaining squamous cell carcinomas.

This subset of tumours predominantly arises from lingual and palatine tonsils within the oropharynx (40-70% of cases), is mainly associated with HPV type 16, and its incidence is increasing in recent years. Patients with HPV-positive tumours tend to be slightly younger than those with HPV-negative tumours, show less alcohol and tobacco intake, present often with more advanced disease stages but seem to have a favourable survival. Furthermore, HPV-positive carcinomas show typical histopathological and molecular characteristics, including poor differentiation grade, basaloid appearance, integrated HPV DNA, wildtype p53, a genetically more stable genome, upregulation of p16INK4A, p14ARF, p21CIP1/WAF1, and downregulation of pRb, cyclin D1 and EGFR. Due to these features, HPV-associated head and neck tumours are considered to be a separate tumour entity. Because of the high correlation between p16INK4A overexpression and HPV presence, p16INK4A is a powerful surrogate marker for HPV-related head and neck cancer and is currently recommended together with HPV-specific (F)ISH or RT-PCR to diagnose a relevant infection and provide prognostic information. Recent studies present more and more evidence that the prognostic advantage for patients with HPV-related tumours is independent of the treatment strategy (surgery, radiotherapy or multimodal). Prospective trials will have to evaluate the most appropriate treatment for this tumour entity.

10.20–11.00 **[S16]** ***Viruses and Lymphoma***

Prof R Jarrett, University of Glasgow

Viruses are associated with a significant minority of human lymphomas. The herpesviruses EBV and HHV-8 and the retrovirus HTLV-I are directly involved in lymphoma pathogenesis, whereas HIV and Hepatitis C virus play indirect roles. Recent reports suggest that the retrovirus XMRV is circulating in the human population and infects lymphocytes but there is no evidence that this virus is detectable in lymphomas. EBV is associated with a range of B and T-cell malignancies, of which EBV-associated Hodgkin lymphoma (HL) is numerically the most important. Most healthy adults are infected by EBV and the virus establishes a reservoir in B-cells. Although EBV is an efficient transforming agent the virus is kept under control by cytotoxic T-cell (CTL) responses. EBV-associated B-NHLs occur where CTL responses are compromised, such as post-transplantation. In these tumours a group of EBV genes associated with growth transformation is generally expressed. More restricted patterns of EBV gene expression are seen in EBV-associated HL and Burkitt's lymphoma. The Hodgkin and Reed-Sternberg (HRS) cells in EBV-associated HL express the EBNA1, LMP-1 and LMP-2 antigens as well as the EBER RNAs and BART miRNAs. HRS cells are B-cells that lack B-cell receptors and LMP-1 and 2 appear to play a role in rescuing these cells from apoptosis, the usual fate of B-cell lacking B-cell receptors. LMP-2 also appears to play a role in the reprogramming of HRS cells. EBV-associated HL is strongly associated with HLA-A alleles and prior history of infectious mononucleosis suggesting that control of EBV infection is also a critical determinant of risk of this disease. In Burkitt's lymphoma, EBNA1 is the only viral antigen expressed by the tumour cells and the role of the virus is less clear. Modern molecular methods of virus discovery are being used to try and identify a virus in EBV-negative HL.

11.00–11.30 **Coffee [FOYER]**

11.30–12.10 **[S17]** ***Viral Infections of the Liver***

Dr R Goldin, Imperial College London

Viral infections of the liver can be divided into those which primarily affect the liver and those which usually involve the liver as part of a systemic illness. The former, especially hepatitis B and C, are important causes of morbidity and mortality worldwide but especially in developing countries. Despite the introduction of increasingly effective therapies for both these viruses, cirrhosis and liver cell cancer remain major problems, even in developed countries. Hepatitis C is now the leading indication for liver transplantation in many of them. The severity of these infections is influenced by co-existing viral infections (especially HIV and, in the case of hepatitis B, delta virus) as well as other co-factors (especially increased alcohol intake and insulin-resistance). Liver biopsies are undertaken to assess the severity of the disease process (in terms of both grade and stage), to look for complications (such as large cell dysplasia), to exclude co-existing liver disease (especially iron overload and fatty change) and to assess disease progression. The importance of extra-hepatic manifestations of viral hepatitis (for example the effect of HCV on the brain) are increasingly being recognised. It should also be noted that, hepatitis E is increasingly being recognised as an important cause of an acute hepatitis in the UK. Viruses involving the liver as part of a systemic illness include cytomegalovirus and he Epstein-Barr virus). The former is more common in patients who are immunosuppressed for example in those who have had liver transplants). The liver disease associated with these viruses is usually mild

12.10–12.50 **[S18]** ***Viral Oncogenesis and Skin Cancers***

Ⓟ Dr C Proby, University of Dundee

Harwood, C[2]; Storey, A[3]; Leigh, I[1]

[1]University of Dundee, United Kingdom; [2]Barts and the London School of Medicine, United Kingdom; [3]Weatherall Institute of Molecular Medicine, Oxford, United Kingdom

Viruses have long been recognised to contribute to a wide spectrum of skin infections. This talk, however, will focus on their putative role in skin carcinogenesis. This is most apparent in immunosuppressed individuals, especially organ transplant recipients (OTR). At Barts & the London NHS Trust we have longitudinally followed a cohort of 900 OTR for over 16 years and documented the skin cancer burden in different ethnic groups. OTR show a 100-fold increased incidence of cutaneous squamous cell carcinoma (cSCC), a 100-fold increase in

appendageal malignancies and a 400-fold increase in Kaposi sarcoma. Human papillomaviruses (HPV), particularly β-papillomaviruses (β-PV), are implicated in cSCC. There is a high prevalence of β-PV DNA in transplant-associated cSCC and studies using in situ hybridisation demonstrate active viral replication within tumours. HPV E6 proteins from diverse HPVs can inhibit UVR-induced apoptosis, providing a mechanism whereby β-PV may promote tumour development in cooperation with the mutagenic effects of UVR. However, these viruses are also ubiquitous in normal skin and hair follicles and, until a hierarchy of risk has been established for β-PV, it seems unlikely that a prophylactic vaccine will be developed. Kaposi sarcoma (KS) is associated with HHV8 infection and is common in OTR from endemic areas such as sub-Saharan Africa. Sixteen cases of KS were observed at our institute, none were HIV-positive. Nine (56%) were African, representing 20% of our African OTRs. KS often presents early with asymmetrical limb swelling. Visceral KS occurred in 4 (25%). Immunosuppression should be reduced or withdrawn and patients switched to m-TOR inhibitors, where appropriate. Three of our KS-OTR required chemotherapy and two were given radiotherapy. When managing transplant-associated KS, control is more feasible than cure.

LECTURE THEATRE Ⓑ

09.00–11.00 ORAL COMMUNICATIONS

Chair: Prof FA Carey, Ninewells Hospital and Medical School, University of Dundee
Prof M Pignatelli, University of Bristol
Categories: Cellular/Molecular; Gastrointestinal

09.00–09.15 [**O35**] ***Quantitative Assessment of Tumour Cell Density Following Preoperative Therapy Compared to Surgery Alone in Rectal Cancer***

Ⓟ West, N[1]; Grabsch, H[1]; Treanor, D[1]; Sebag-Montefiore, D[2]; Thorpe, H[3]; Jayne, D[2]; Rutten, H[4]; Swellengrebel, H[5]; Nagtegaal, I[6]; Quirke, P[1]

[1]Leeds Institute of Molecular Medicine, United Kingdom; [2]Leeds Teaching Hospitals Trust, United Kingdom; [3]University of Leeds, United Kingdom; [4]Department of Surgery, Netherlands; [5]Netherlands Cancer Institute, Netherlands; [6]Department of Pathology, Netherlands

Patients with rectal cancer may be offered preoperative therapy. Regimens differ and include short course radiotherapy (25Gy) or long course chemoradiotherapy (CRT). The response to therapy is usually assessed by the pathologist on the resection specimen using one of several subjective tumour regression grading systems with poor reproducibility. We report here an objective quantitative tumour cell density (TCD) analysis allowing direct comparison of different regimens.
The glass slides from four rectal cancer series were digitally scanned: surgery alone (controls), 25Gy with immediate surgery, 50Gy with capecitabine (50Gy+Cap), and 45Gy with capecitabine and oxaliplatin (45Gy+Cap/Ox). Using a digital slide viewer, a 9mm^2 area was selected in the region of greatest TCD and a systematic random sample of 300 points was superimposed using virtual graticule software. TCD was expressed as the percentage of points falling on tumour cells out of all the informative points.
The running mean TCD showed stabilisation after approximately 30 cases. There was a significant reduction in median TCD in all three groups when compared to the controls (see table). There was a significant difference in TCD between the 50Gy+Cap and the 45Gy+Cap/Ox groups (p=0.043).
Quantitative TCD analysis gives an objective measure of the response to preoperative therapy in rectal cancer. 25Gy significantly reduced the TCD after early resection. Long course CRT with 6 weeks delay leads to a significantly greater reduction. The addition of oxaliplatin appeared to further reduce the TCD when compared to capecitabine alone as radiosensitizer. This method may offer a reproducible quantifiable solution to identifying the effectiveness of different preoperative regimens.

Regimen	N	Median TCD (%)	IQR (%)	P value vs. control
Controls	45	56	50 - 63	-
25Gy	45	36	14 - 46	<0.0001
50Gy+Cap	45	14	2 - 22	<0.0001
45Gy+Cap/Ox	45	4	1 - 13	<0.0001

09.15–09.30 [**O36**] ***Lymph Node Staging According to TNM 7th ed. Improves Prognostic Stratification of Patients with Oesophageal Cancer***

Ⓟ Jose, P[1]; Mirza, A[2]; Pritchard, S[2]; Hayden, J[3]; Grabsch, H[1]

[1]Pathology&Tumour Biology, Leeds Institute of Molecular Medicine, United Kingdom; [2]Dept of Histopathology, University Hospital of South Manchester, United Kingdom; [3]Dept of Upper Gastrointestinal & Minimally Invasive Surgery, Leeds Teaching Hospitals NHS Trust, United Kingdom

TNM classification is the gold standard for predicting patient prognosis after surgery. TNM7 has been recently published, stratifying oesophageal cancer patients on the basis of the number of involved lymph nodes and introducing two separate categories for early oesophageal cancer (pT1a and pT1b). We examined whether TNM7 allows better prognosis prediction in a series of neoadjuvantly treated oesophageal cancer patients from two different hospitals.
Clinicopathological data from 255 patients who had surgery after neoadjuvant chemotherapy were analysed retrospectively. Median age was 62 ys (range: 35 to 80ys), 74% were male, 78% were adenocarcinomas. Median

lymph node harvest was 30 nodes (range: 3 to 104); median follow up time was 1.5 ys (range: 0.08 to 9 years). Prognostic value of staging according to TNM6 and TNM7 was compared.
TNM6 ypT and ypN were both independently related to patients survival (p=0.03 and p<0.001, respectively). TNM7 ypN was associated with patient prognosis (p<0.001) identifying three prognostically different subgroups (node negative vs. 1 to 6 positive nodes vs. more than 6 positive nodes). TNM7 ypT was not related to patient survival (p=0.053) and no prognostic difference was seen between intramucosal (pT1a) and submucosal (pT1b) cancer.
Whilst the TNM7 ypN stage after chemotherapy provides new distinct prognostic subgroups, it remains to be shown whether the introduction of these new prognostic subgroups will lead to changes in patient management in the future. The TNM7 subclassification of early cancers failed to produce prognostically different subgroups in our series which could be related to the relatively small number of patients in the ypT1 category. However, TNM classification categories are based on data from surgery alone treated patients. Patient's prognosis with pT1 stage after neadjuvant chemotherapy may be different from that of pT1 after surgery alone.

09.30–09.45 [**O37**] ***Field Cancerization in Crohn's Disease***

Ⓟ Graham, T[1]; Galandiuk, S[2]; McDonald, S[3]; Leedham, S[4]; Wright, N[3]

[1]Cancer Research UK, London, United Kingdom; [2]University of Louisville, United States; [3]Barts and the London School of Medicine and Dentistry, United Kingdom; [4]Oxford University, United Kingdom

Purpose To determine the mechanism of tumorigenesis in Crohn's disease (CD) associated cancers. In Ulcerative Colitis, inflammation and tissue repair by crypt fission produces a field of clonally expanded, genetically abnormal crypts that predispose towards multifocal neoplasia development – field cancerization. We completed a clonal ordering study in a Crohn's colitis patient to investigate whether CD-associated cancers can follow a similar pattern. Methods Biopsies and resection material from throughout the colon and terminal ileum were collected from a single Crohn's pan-colitis patient over 8 years (1996-2004), during which two cancers and multiple dysplastic lesions developed. 214 crypts were microdissected and their mutation burden (PTEN, p53, APC, K-ras, CTNNB1 point mutations and LOH at 3p(FHIT), 9p(p16), 5q(APC), 17p(p53),18q(SMAD4)) was assessed. Phylogenetic trees were constructed for each neoplastic lesion. Results Non-dysplastic mucosa from the rectum and sigmoid pre-2000 contained no detected mutations. A rectal cancer removed in 2000 contained a p53 c.713G>A mutation only in a subclone. In 2001, phenotypically non-dysplastic crypts from the rectum and dysplastic crypts in the sigmoid had a different p53 c.742C>T mutation. By 2004, the same p53 c.742C>T mutation was detected in rectum, sigmoid, transverse, descending colon and terminal ileum, indicating it had crossed the ileal-caecal valve, demonstrating widespread expansion of the p53 mutant clone. Multi-focal neoplasia arose within this mutant field: dysplastic lesions in the ascending colon and terminal ileum, and a rectal cancer, all had p53 c.742C>T as their apparent initiating mutation. Conclusions Marked field cancerization underlies tumorigenesis in this Crohn's patient. In four years a clone expanded from the rectum, along the entire colon into the small bowel. Non-dysplastic crypts can carry a biologically significant mutation burd

09.45–10.00 [**O38**] ***Mismatch Repair Status in Stage II Colorectal Cancer : Substantially Reduced Recurrence Rate In MMR Deficient Tumours Supports Routine Testing***

Ⓟ Hutchins, G[1]; Southward, K[1]; Handley, K[2]; Gray, R[2]; Magill, L[2]; Beaumont, C[1]; Stahlschmidt, J[3]; Richman, S[1]; Seymour, M[3]; Kerr, D[4]; Quirke, P[1]

[1]Leeds Institute of Molecular Medicine, United Kingdom; [2]Birmingham Clinical Trials Unit, University of Birmingham, United Kingdom; [3]Leeds Teaching Hospitals NHS Trust, United Kingdom; [4]Sidra Medical and Research Centre, Doha, Qatar

Adjuvant therapy in stage II colorectal cancer (CRC) is limited to patients with high recurrence risk. Currently, no consistent method exists to identify them. CRCs displaying defective DNA mismatch repair (dMMR) are reported in multiple small studies to be of low recurrence risk. Despite this, MMR testing is not routine. Validation of the prognostic utility of MMR status in larger studies is needed to define clinical utility. Additionally, assessment of the predictive capacity of MMR status in large randomised studies is required in view of reported attenuated clinical outcomes in dMMR patients treated with adjuvant chemotherapy. Here we report the prognostic and predictive properties of DNA MMR status using material from QUASAR-1, a large randomised controlled trial that demonstrated chemotherapeutic benefit from 5-fluoruracil (5-FU) in stage II colorectal cancer.
1915 cases (91% stage II colorectal cancer) were analysed for defective mismatch repair status by immunohistochemistry using antibodies against MLH1 and MSH2. Patient outcomes by MMR status were analysed by logrank methods using time to recurrence as the primary endpoint.
The large majority of dMMR tumours were in proximal colon. Recurrence risk was halved in tumours displaying mismatch repair defects in both all patients (Risk ratio (RR)= 0.53, 95%CI = 0.40 to 0.70: p<0.0001) and right-sided stage II tumours only (RR = 0.44, 95%CI = 0.29 to 0.67; p<0.0001). Attenuation of recurrence risk in right-sided dMMR stage II tumours was equivalent in the presence and absence of chemotherapy.
Routine testing of MMR status is justified in sporadic stage II colorectal cancer to allow identification of low recurrence risk. There was no evidence to support the hypothesis that chemotherapy treated dMMR patients have poor outcome.

10.00–10.15 [**O39**] ***Improving the Quality of Colon Cancer Surgery Through a Multidisciplinary Education Programme: Early Results***

West, N[1]; Ⓟ Sutton, K[1]; Ingeholm, P[2]; Hohenberger, W[3]; Quirke, P[1]

[1]Leeds Institute of Molecular Medicine, United Kingdom; [2]Hillerød Hospital, Copenhagen, Denmark; [3]University Hospital of Erlangen, Germany

The importance of the plane of rectal cancer surgery is well established, however, the evidence for a similar effect in colon cancer is limited. We have previously reported better outcomes with mesocolic plane surgery and shown that complete mesocolic excision with central vascular ligation (CME & CVL) produces an oncologically superior specimen.

We received specimen photographs and clinicopathological data from a series of 280 primary resections for colon cancer; 99 from surgeons trained in CME & CVL and 181 from surgeons prior to training. The plane of dissection was graded using a method described previously. Tissue morphometry was performed using ImageScope v10 (Aperio, CA).

CME & CVL surgeons were more likely to operate in the mesocolic plane (74% vs. 37%, $p<0.0001$) and remove more lymph nodes per specimen (median 27 vs. 19, $p<0.0001$). 123 fresh and 145 fixed specimen photographs were suitable for morphometry. CME & CVL surgeons removed more tissue longitudinally in both the fresh (median 316 vs. 266mm, $p<0.0001$) and fixed (271 vs. 208mm, $p<0.0001$) specimens, and centrally between the tumour and the high vascular tie in both fresh (104 vs. 89mm, $p=0.033$) and fixed (82 vs. 65 mm, $p=0.001$).

We have shown that surgeons trained in CME & CVL are more likely to operate in the mesocolic plane, remove more tissue centrally and longitudinally, and achieve greater lymph node yields. This provides further evidence for the oncological superiority of CME & CVL and demonstrates that surgical education can directly influence the quality of the specimen produced.

10.15–10.30 [**O40**] ***Pulse Chase Experiments Using IUdR Identifies Barrett's Label-Retaining Stem Cells.***

Ⓟ Nicholson, A[1]; Harrison, L[2]; Barr, H[3]; Graham, T[4]; McDonald, S[1]; Wright, N[1]; Harrison, R[5]; Jankowski, J[1]

[1]Barts and the London, United Kingdom; [2]Digestive Diseases, Leicester, United Kingdom; [3]Gloucester Royal, United Kingdom; [4]Cancer Research UK, London, United Kingdom; [5]Leicester Royal Infirmary, United Kingdom

Background. A gastro-intestinal epithelial cell will be shed from the epithelial surface into the lumen and be replaced from below by the progeny of a stem cell (SC). SC's are thought to be the target for mutations that may lead to the aberrant epithelial biology seen Barrett's Metaplasia (BM). To date the location of putative Barrett's metaplastic stem cell (BMSC) within the epithelium of BM, is unknown. *Methods.* We used iododeoxyuridine (IUdR), which is incorporated into the DNA of replicating cells and when retained for prolonged periods acts as a marker to identify the label retaining cells (LRC). This method has been used previously to identify stem cells in other tissues. Four patients with BM undergoing planned oesophagectomy were infused with IudR at intervals prior to their surgery (7-67 days). After resection, tissue samples of normal oesophagus, BM, normal stomach and tumour were extracted for immunocytochemistry and FACS. Finally we made a model of the division rate of cells. *Results.* LRCs were seen in the basal layer of the squamous epithelium at all pulse chase times. LRCs were seen at the neck/foveolar regions of the gastric tissue at 11 days conforming to the suggested gastric SC zone. LRC's were seen at the base of the Barrett's gland up to 67 days. Furthermore a model of the cell turn-over in the base of Barrett's glands show cells cycle every day. Preliminary results using putative GI SC markers in Barrett's show *in situ* LGR5 expression to be at the base of the Barrett's gland in these patients. *Conclusion.* This is the first report of LRC's in the human oesophagus, our findings correlate with the hypothesised location of SCs in squamous and gastric mucosa. LRCs were located in the base of the BM gland implying that this is the location of BMSCs. Analysis and characterisation of the BMSCs and their niche are underway, involving the use of other GI SC markers and their co-localisation with IuDR.

10.30–10.45 [**O41**] ***CD24 Enhances Cell Migration and Invasion in Colorectal Cancer Through Akt Pathway Activation***

Ⓟ Ahmed, M; Kindle, K; Jackson, D; Aleskandarany, M; Ilyas, M

Division of Pathology, Queen's Medical Centre, University of Nottingham, United Kingdom

CD24 has been found to be over-expressed in several types of solid tumours and has usually been linked to poor prognosis and diverse biological effects. However, the cellular mechanisms of CD24-mediated effects are still unclear. We studied the function of CD24 in colorectal cancer (CRC) cell lines to scrutinize its cellular effects and possible downstream signalling pathways.

Methods: The expression of CD24 was assessed by q-RT-PCR and western blotting. CD24 was functionally evaluated by forced expression in HCT116 using CD24 expression plasmid and knockdown, by RNA interference, in HT29 CRC cell lines. Cellular migratory and invasive characters were assessed using trans-well migration, matrigel invasion and wounding assay. The effects on colony formation were assessed in soft agar. Human phospho-kinase antibody array involving 46 kinases (R&D systems) was used to investigate the potential signalling pathways of CD24-mediated effects in GP2D cell line.

Results: Forced expression of CD24 resulted in increased colony formation($p<0.01$) compared to control, and increased migratory and invasive capacities, that were decreased after CD24 knockdown($p<0.01$). Knockdown of CD24 was associated with marked reduction of pAkt(S473) activation of by 62.1 % compared to control, along with its upstream activator pFAK(Y397) by 47%. Downstream to Akt, there were reduction in P27(T198) by 93.6 %,

peNOS(S1177) by 71.9 % and CREB(S133) by 49 %.
Conclusions: Expression of CD24 increased the migratory and invasive capacities of CRC cell lines and enhanced colony formation in soft agar; features associated with high metastatic potential and increased cellular tumorigenicity. We were able to show that CD24-induced effects are at least in part through Akt signalling pathway. Our findings would give an insight that targeting CD24 early in CRC patients would deactivate Akt signalling, hence abolishing its adverse biologic effects.

10.45–11.00 [**O42**] ***Frequency of KRAS Mutations Differs Significantly Between Colorectal Cancer Originating from Nigeria and UK***

Ⓟ Richman, S[1]; Chambers, P[1]; Sanni, L[2]; Hemmings, G[1]; Rotimi, O[2]; Abdulkareem, F[3]; Grabsch, H[1]; Quirke, P[1]

[1]Leeds Institute of Molecular Medicine, United Kingdom; [2]Histopathology, St James University Hospital, Leeds, United Kingdom; [3]Morbid Anatomy Dept, Lagos, Nigeria

Background: Activating mutations in KRAS codons 12, 13 or 61 occur in up to 50% of colorectal cancers resulting in EGFR-independent cell signalling through the MAPK pathway. BRAF, located downstream of KRAS, can be activated by a mutation in codon 600. In a previously published study on patients in the MRC FOCUS trial, mutation in either KRAS or BRAF was associated with poor prognosis. Aim: To compare frequency and type of KRAS and BRAF mutation between Nigerian colorectal cancer (CRC) and CRC from the MRC FOCUS trial. Methods: DNA was extracted from 200 formalin-fixed, paraffin embedded Nigerian CRC and KRAS codons 12, 13 and 61 and BRAF codon 600 were assessed by pyrosequencing. Mutation rates and the spectra were determined. Results: Pyrosequencing was successful in 112 Nigerian samples for all KRAS and BRAF codons. Mutations in KRAS codons 12 and 13 were detected in 23/112 (21%) of samples. All KRAS codon 61 all samples showed a WT genotype. 5% of samples carried a mutation in BRAF codon 600. Mutation frequency of KRAS but not BRAF differs between the Nigerian and the FOCUS trial ($p<0.0001$). Interestingly, although KRAS mutation frequency was lower in the Nigerian CRC, the mutation spectra were very similar. Conclusion: The Nigerian and FOCUS trial population differ significantly in KRAS mutation frequencies, but have similar mutation spectra. Nigerian patients may have a greater benefit from anti-EGFr antibody therapy than Caucasian populations.

11.00–11.30 Coffee [FOYER]

LECTURE THEATRE Ⓑ

11.30–13.00 ORAL COMMUNICATIONS

Chair: Dr MJ Arends, University of Cambridge, Addenbrooke's Hospital
Dr RFT McMahon, University of Manchester
Categories: Education and Audit; Gastrointestinal

11.30–11.45 [**O43**] ***Audit of Oral Mucosal Biopsies: Size and Orientation at Cut-up Influencing Ease of Diagnosis***

Ⓟ Mukherjee, A; Kendall, C

Department of Histopathology, University Hospitals of Leicester, United Kingdom

Purpose of the study: The size and orientation of oral mucosal biopsies are crucial factors influencing diagnosis. Local guidelines state that, for sites permitting sufficient depth (eg. tongue, buccal mucosa), an adequate biopsy would be 4mm deep, halved longitudinally at cut-up and embedded on cut surface. In contrast, for sites that yield shallow biopsies (eg. gingiva), the average depth would be 2mm and the whole specimen should be embedded on edge. This audit aimed to determine whether local guidelines were being followed and influences on diagnosis, if any.
Methods: 101 oral mucosal biopsies processed during a year were selected through SNOMED search. The size of the biopsy, presence of crush artefact and orientation at cut-up were assessed by review of slides by two independent pathologists. The influence of surgical parameters and pathological cut-up on diagnosis were assessed qualitatively.
Summary of results: deviations from guidelines occurred at surgical biopsy stage for 49% Benign (B) and 40% Malignant (M) cases and at pathology cut-up stage for 26% (B) and 16% (M) Cases. The deviations at surgical biopsy included crush artefact, inappropriate size and number. Deviations at cut-up included inappropriate orientation and sub-optimal halving. In 6 cases, the compromise at surgery prompted deviation at pathology cut-up. The ease of diagnosis was compromised in only 20.8% Cases, including 2 malignant ones. However, a re-biopsy was not necessary in any case.
Conclusions: We conclude that strict adherence to guidelines and effective dialogue between surgeons and pathologists will prove beneficial for ease of diagnosis.

11.45–12.00 [**O44**] ***Analysis of the Clonality of Barrett's Oesophagus Glands Reveals They are Clonal Units and Establishes a Common Stem Cell for Glandular and Squamous Epithelium.***

Ⓟ Nicholson, A[1]; Graham, T[2]; Simpson, A[1]; Humphries, A[2]; Wright, N[1]; McDonald, S[1]; Jankowski, J[1]

[1]Barts and the London, United Kingdom; [2]Cancer Research UK, London, United Kingdom

Introduction. Barrett's oesophagus (BO) describes the change from the squamous epithelium of the oesophagus to an intestinal columnar phenotype. Little is known about the origin and stem cell architecture of BO glands, or about the mechanism of gland spread. We have used non-pathogenic mitochondrial DNA (mtDNA) mutations as a marker of clonal expansion to investigate this within BO.
Methods. We used histochemistry to detect cytochrome-c-oxidase (CCO)-deficient glands, a mitochondrially-encoded enzyme, likely to have mtDNA mutations. Laser capture microdissection was used to isolate cells. Proteinase K digestion was used to extract DNA. Mutations were identified by nested PCR and sequencing of the entire mtDNA genome. Immunohistochemistry was used to show differentiated cell lineages and mitochondrial activity.
Results. CCO-deficient glands were seen in metaplastic sections. Deficient glands were shown to have normal mt activity and contain fully differentiated cells lineages. All cells within a gland contained the same mutation, indicating that glands are clonal units. Patches of adjacent CCO-deficient glands, consisting of between 2-7 glands, containing a common mutation were seen. This suggests that BO glands spread by fission. In one case, cells from squamous tissue shared the same mtDNA mutation as cells from underlying glandular epithelium, suggesting the two tissue compartments are derived from a common progenitor cell.
Conclusions. We have shown that Barrett's glands are clonal units. Where a single stem cell with a mtDNA muation has taken over the entire stem cell population of a metaplastic gland (niche succession) resulting in monoclonal conversion to a wholly deficient gland. Patches of adjacent glands share the same mtDNA mutation, indicating that BO glands spread by fission. Finally we have shown that both glandular tissue and squamous epithelium can be derived from a common progenitor cell.

12.00–12.15 [**O45**] ***Analysis of Cross-Sections of Oesophagectomy Specimens is Essential to Assess Variation in Surgery***

Ⓟ Jose, P[1]; Hayden, J[2]; Dexter, S[2]; Sarela, A[2]; Sue-Ling, H[2]; Grabsch, H[1]

[1]Pathology & Tumour Biology, Leeds Institute of Molecular Medicine, United Kingdom; [2]Dept of Upper Gastrointestinal & Minimally Invasive Surgery, Leeds Teaching Hospitals NHS Trust, United Kingdom

There is currently no consensus regarding extent of resection for oesophageal cancer. In our specialist cancer centre, all surgeons perform a "radical oesophagogastrectomy with 2-field lymphadenectomy". The aim of our pilot study was to establish (i) the feasibility of morphometric measurements in macroscopic resection specimens, (ii) whether there is a relationship between the extent of resection, post-operative morbidity and patient survival.
138 oesophageal resection specimens performed by three surgeons from one specialist cancer centre were investigated. The area of perioesophageal tissue and length of exposed or disrupted muscularis propria at the circumferential resection margin were measured on macroscopic pictures after cross-sectioning the specimen. Intraobserver variation was assessed. Relationship of morphometric measurements, surgeons and clinicopathological data including survival was analysed.
Reproducibility of measurements was confirmed. The area of resected perioesophageal tissue varied between specimens (median: 495 mm^2, range: 87-1340 mm^2) and surgeons ($p<0.001$) and was related to lymph node yield (median: 32 nodes, range 4-104 nodes, $p<0.001$). There was neither a significant relationship between the area of perioesophageal tissue and post-operative morbidity nor with survival.
Our pilot study demonstrated objectively that the extent of perioesophageal resection varied between surgeons for the same procedure. Extended perioesophageal resection improved lymph node staging without compromising post-operative morbidity. Further work in a larger series is warranted to validate our results and to establish whether there is a survival benefit from a more 'extended' resection. This important work will only be possible if cross-sectioning and photographing of oesophagectomy specimens is widely practised in pathology departments.

12.15–12.30 [**O46**] ***CEACAM6, A Novel Biomarker in Gastric Cancer.***

Ⓟ Sivakumar, S; Pollock, S; Speirs, V; Grabsch, H

Pathology & Tumour Biology, Leeds Institute of Molecular Medicine, United Kingdom

CEACAM6 is a cell surface protein that functions as adhesion molecule and can induce resistance to apoptosis. CEACAM6 overexpression has been related to poor prognosis in breast, colorectal and pancreatic cancer. There are currently no data available at all regarding frequency of CEACAM6 protein expression and relationship to clinicopathological data and patient survival in gastric cancer (GC).
CEACAM6 expression was assessed in 481 GC using tissue microarrays and immunohistochemistry. The percentage of positive tumour cells/case was established. Prominent intratumoural heterogeneity was noted, often with one core being completely negative whereas the second core from the same case showed 100% positive tumour cells. In view of the aim to identify a new biomarker, we decided to use the highest value/case for further analyses and did not average scores from all cores/case. A modified ROC curve approach identified a cut off of 50% positive tumour cells as most sensitive and specific to predict relationship with survival.
CEACAM6 staining was found to be cytosolic with some membranous accentuation. Median percentage of CEACAM6 positive cells: 90 (range: 0 to 100). 20% GC were CEACAM6 negative and in 48% GC all tumour cells were positive. High CEACAM6 expression was more common in diffuse type GC ($p=0.039$), high pT stage ($p=0.020$) and related to poor patient survival ($p=0.033$, univariate analysis).
This is the first study to report the frequency of CEACAM6 expression in GC. 80% of GC were CEACAM6 positive, a frequency similar to that reported in pancreatic cancer. Beyond its prognostic value and relationship to tumour morphology, CEACAM6 could thus be a promising new therapeutic target in GC and preclinical assessment of already existing therapeutic anti-CEACAM6 antibodies in GC models should be considered.

12.30–12.45 [**O47**] ***The Selective Use of Levels in Gastrointestinal Histopathology Does Not Compromise the Diagnosis of Gastro-oesophageal Malignancy***

Ⓟ West, K[1]; Salam, R[2]; McGregor, A[3]; Bartlett, H[2]

[1]Dept of Cellular Pathology, Leicester, United Kingdom; [2]University of Leicester Medical School, United Kingdom; [3]University Hospitals of Leicester, United Kingdom

In 2006, following an audit the routine cutting of levels was abandoned in our department. Concerns were raised that this may result in missing cases of malignancy where a scanty infiltrate was present. All biopsies from the oesophagus and stomach coded as malignant from the time period Jan 2007 – March 2009 were identified. The specimen records were then reviewed in order to determine whether or not any previous relevant biopsies had been submitted since the change to selective levels. All previous biopsy records were assessed to see if levels had been examined. 400 cases of upper GI malignancy were identified. Thirty-seven patients (9.3%) had undergone a total of 51 previous biopsies; 50/51 had levels examined and were all correctly reported as showing no definite evidence of malignancy. In 1/51 cases levels were not examined. Further sections subsequently confirmed the absence of cancer No upper gastro-oesophageal malignancies were missed as a result of the policy of selective requesting of levels. Concerns over missing a diagnosis of malignancy are not justified. The recommendation that levels should be examined routinely should not be perpetuated as it is not evidence-based.

12.45–13.00 [**O48**] ***Lymphocytic Infiltration in Colorectal Cancer and its Relationship to Mismatch Repair Deficiency and the Systemic Neutrophil-to-Lymphocyte Ratio***

Ⓟ Pine, J[1]; West, N[1]; Hutchins, G[1]; Jayne, D[2]; Prasad, K[1]; Quirke, P[3]

[1]St James's University Hospital, Leeds, United Kingdom; [2]Leeds General Infirmary, Leeds, United Kingdom; [3]University of Leeds/St James's University Hospital, Leeds, United Kingdom

Introduction: Colorectal cancers (CRC) induce an inflammatory/immune response both systemically and in local tumour stroma. A pronounced lymphocytic infiltrate correlates with better prognosis. We've shown that inflammation, measured by neutrophil/lymphocyte ratio (NLR) in the peripheral blood, predicts outcome in CRC. We examined the association between local tumour lymphocytic infiltrate, mismatch repair deficiency (dMMR), NLR and outcome. Method: Patients who had bowel resection for CRC at the Leeds Teaching Hospitals from 2000 to 2004 were identified. H&E sections were cut and scored 1 to 3 (mild to severe) based on lymphocytic reaction to tumour both in the tumour centre and invasive margin. The scoring system was based on Klintrup et al (2005). Two independent observers scored the slides to assess inter-observer agreement. Results: 358 patients were scored for tumour centre and invasive margin. Only invasive margin demonstrated significance. At the invasive margin the reaction was graded mild 68.4%, moderate 29.3% and severe 2.3%. The grade of lymphocytic infiltration at the margin was significant for overall (p=0.001) and disease-free (p=0.027) survival with a moderate/severe reaction corresponding to improved outcome. A moderate/severe lymphocytic reaction was associated with lower stage (p=0.001), less vascular invasion (p=0.01), dMMR (p=0.04) and lower NLR (p=0.005). Discussion: Intratumoral lymphocytes had no effect whilst those at the invasive margin conferred a survival advantage. A moderate/severe reaction at the margin was associated with less advanced cancer and dMMR. This may be due to a more immunogenic tumour or an indication that the tumour has yet to develop features to allow it to escape detection by the adaptive immune system. NLR measures systemic inflammation and there appears to be an association between NLR and peritumoral lymphocytosis. Further work is needed to determine mechanistic links.

FOYER

13.00–14.00 LUNCH AND TRADE EXHIBITION

LECTURE THEATRE Ⓑ

13.30–14.30 PATHOLOGICAL SOCIETY ANNUAL BUSINESS MEETING

Members will be sent an Agenda

FOYER

14.00–15.00 POSTER VIEWING AND CHAIRMAN'S ROUNDS *(Tea/coffee will be served)*

CATEGORY	POSTER NUMBERS
Gastrointestinal	P83–P103[1]
Genitourinary/Renal	P104–P111[2]
Gynaecological	P112–P116[2]
Hepatobiliary/Pancreas	P117–P121[3]

Chair: [1] Dr RFT McMahon, Manchester and Prof M Pignatelli, Bristol
[2] Dr ISD Roberts, Oxford
[3] Dr KP West, Leicester

LECTURE THEATRE Ⓑ

15.00–16.00 **SLIDE SEMINAR CASE REVIEW: *Head/Neck/Oral Pathology***

Chair: Dr G Stenhouse, Ninewells Hospital and Medical School, Dundee

Contributors:

Dr G Stenhouse

Dr S White, Dundee Dental School, University of Dundee

LECTURE THEATRE Ⓐ

16.00–17.30 **PLENARY ORAL SESSION**

Chair: Prof IO Ellis, University of Nottingham

Prof CS Herrington, University of St Andrews

16.00–16.15 [**PL1**] ***The First Mouse Model Phenocopying Human Fanconi Anaemia Provides Novel Insight into the Molecular Defect and Pathogenesis of the Disease***

Ⓟ Crossan, G[1]; Van Der Weyden, L[2]; Arends, M[3]; Adams, D[2]; Patel, K[1]

[1]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom; [2]Wellcome Trust Sanger Institute, Cambridge, United Kingdom; [3]University of Cambridge, Addenbrooke's Hospital, United Kingdom

Fanconi Anaemia is a rare, genetically complex, recessive condition characterized by congenital abnormalities, progressive bone marrow failure and cancer predisposition. At the molecular level Fanconi Anaemia is characterized by spontaneous chromosomal aberrations and extreme sensitivity to DNA crosslinking agents exemplified by the chemotherapeutic agent cisplatinum. DNA interstrand crosslinks pose profoundly toxic lesions to cells blocking both transcription and replication. The genes disrupted in Fanconi Anaemia comprise a pathway to repair these lesions. In order to further define the genetic basis of Fanconi Anaemia we have created an Slx4 deficient mouse model. This model reveals the requirement of Slx4 in the repair of interstrand crosslinks. We show that the interaction of Slx4 with the known endonuclease, Xpf, is required for repair. In addition Slx4 segregates the role of Xpf in interstrand crosslink repair from its function in nucleotide excision repair. The Slx4 deficient mouse provides the first model of Fanonci Anaemia to recapitulate the human disease process. Slx4 deficient mice are sterile and growth retarded. There is increased lethality within the first 3 months of life explained by an increased incidence of congenital abnormalities and haematopoeitic dysfunction. Slx4 deficient mice also recapiltuate other aspects of the human disease with an increased incidence of eye abnormalities and endocrine dysfunction. Together these data reveal Slx4 to be a novel component of the Fanconi Anaemia pathway segregating the function of a key endonuclease to crosslink repair. This provides genetic evidence linking the Fanconi pathway directly to the endonuclease mediated incision step of crosslink repair. This also provides us with the first mouse model phenocopying human Fanconi Anaemia providing new insights into the pathogenesis of this human condition.

16.15–16.30 [**PL2**] ***Reduced Expression of the Tumour Suppressor Gene* LKB1 *in a Subset of Osteosarcomas***

Ⓟ Duhamel, L[1]; Damato, S[2]; Halai, D[3]; Shalaby, A[4]; Ye, H[3]; Idowu, B[3]; Eskandarpour, M[1]; Presneau, N[1]; Flanagan, A[1]

[1] Department of Pathology, Cancer Institute, University College London, United Kingdom; [2]Histopathology Department, Whittington Hospital NHS Trust, London, United Kingdom; [3]Department of Histopathology, Royal National Orthopaedic Hospital, Stanmore, United Kingdom; [4]Institute of Orthopaedics and Musculoskeletal Science, University College London, United Kingdom

The report that *LKB1*-deficient mice develop bone-forming tumours begs the question whether loss of this tumour suppressor gene, which is associated with Peutz-Jehger syndrome, plays a role in the pathogenesis of human osteosarcomas (OS).

Western blot demonstrated that 70% of primary OS (15/21) and 16% of OS cell lines (2/12) had very low or undetectable levels of LKB1 protein. LKB1 immunohistochemistry confirmed the western data with analysis of tissue microarrays (TMAs) showing 153/259 primary OS (59%) with very low or no immunoreactivity. Among this cohort, 52/68 (76%) good and 60/104 (58%) poor responders to chemotherapy did not express LKB1 ($p=0.0085$, one-tailed Fisher's exact test). Concomitant Akt-activation of TCS2, via the phosphorylation of T1462, was present on 52/137 LKB1-negative cases compared to 80/97 LKB1-positive cases ($p<0.0001$).

No copy number loss of the *LKB1* region was identified in 93 OS by interphase fluorescent *in situ* hybridisation. Direct sequencing of the 21 OS failed to detect *LKB1* mutations in the exons commonly reported to harbour mutations. The same samples showed that 4/12 informative cases had lost one parental allele at the locus of a single nucleotide polymorphism, rs34928889. Finally, all 21 OS expressed significant *LKB1* mRNA levels as assessed by qRT-PCR. Only 1/15 OS cell lines revealed low levels of *LKB1* mRNA implying the mechanism of LKB1 protein loss in this cell line is different than that in the primary tumours. Preliminary *in silico* analysis of our microRNA OS data suggests that low LKB1 protein expression is unlikely to be accounted for by microRNA directly targeting *LKB1* mRNA.

To conclude, OS can be classified by their LKB1 protein expression, and LKB1 loss is associated with a good response to chemotherapy. The data imply that LKB1 loss is due to post-transcriptional event(s). This finding gives new insights on the pathogenesis of bone tumours.

16.30–16.45 [**PL3**] ***The Role of the Sister Chromatid Cohesion Establishment Factor, ChlR1 in DNA Damage Repair***

Ⓟ Feeney, K; Wasson, C; Parish, J

University of St Andrews, United Kingdom

ChlR1 is an ATP dependent DNA helicase involved in the establishment of sister chromatid cohesion during DNA replication. Heritable defects in sister chromatid cohesion have been linked to several diseases such as Cornelia de Lange and Roberts Syndrome. Similarly, mutations in ChlR1 have been linked to Warsaw Breakage Syndrome, a newly identified cohesinopathy characterised by severe microcephaly, growth retardation and abnormal skin pigmentation. Cells derived from an affected individual display drug-induced chromosomal breakage and sister chromatid cohesion defects, indicating that ChlR1 may function at the interface between DNA repair and sister chromatid cohesion.

In order to determine the function of ChlR1 in DNA damage repair, we use alkaline and neutral comet assays. We show that ChlR1 depletion causes a significant defect in DNA damage repair. To further define the mechanism and ChlR1-dependent DNA damage repair, we use tandem affinity purification to isolate ChlR1 associated protein complexes. These studies have isolated DNA-PKcs as a novel ChlR1 interacting protein. Furthermore, we show that the interaction between ChlR1 and DNA-PKcs is enhanced following gamma radiation. DNA-PKcs is essential for DNA repair via the non-homologous end joining (NHEJ) pathway. DNA-PKcs is recruited to double strand breaks (DSBs) and subsequently recruits a number of other proteins to facilitate repair. DNA-PKcs then stimulates the activity of a number of these proteins by phosphorylating them and we hypothesise that ChlR1 is recruited to sites of DNA damage by DNA-PKcs and may be phosphorylated to induce cohesion establishment at DSBs.

We have also isolated the DNA replication factors MCM7 and PCNA as ChlR1 interacting proteins. Stalled replication forks account for a large percentage of DSB and we hypothesise that ChlR1 may be involved in restarting of stalled replication forks as cohesin rings are passed during replication.

16.45–17.00 [**PL4**] ***Targeting DNA Replication Before it Starts: Cdc7 as a Therapeutic Target in p53-Mutant Breast Cancer***

Ⓟ Proctor, I[1]; Rodriguez-Acebes, S[1]; Loddo, M[1]; Wollenschlaeger, A[1]; Falzon, M[2]; Prevost, A[3]; Sainsbury, R[1]; Williams, G[1]; Stoeber, K[1]

[1]University College London, United Kingdom; [2]University College London Hospital, United Kingdom; [3]King's College London, United Kingdom

Current treatment options for Her2-overexpressing (ER-/PR-/Her2+) and triple-receptor negative (ER-/PR-/Her2-) breast cancers are limited and often harmful, and metastatic disease remains incurable. Targeting of growth signaling networks is often constrained by pathway redundancy or growth-independent cancer cell cycles. Cdc7 regulates entry into S phase by promoting DNA replication. This essential kinase acts as a convergence point for upstream growth signaling pathways and has been identified as a potential anti-cancer target.

We show that increased Cdc7 expression during mammary tumourigenesis is significantly associated with Her2-overexpressing (Her2) and triple-negative (TN) tumour subtypes, accelerated cell cycle progression, arrested tumour differentiation, genomic instability and reduced disease-free survival, thus implicating its deregulation in the development of aggressive disease. By inhibiting Cdc7 with small interfering RNAs, we demonstrate that p53-mutant Her2 and TN breast cancer cell lines enter an abortive S phase followed by apoptotic cell death. In contrast untransformed breast epithelial cells avoid lethal S phase entry by activating a p53-dependent Cdc7-inhibition checkpoint. Importantly, this G1 arrest in normal cells is rapidly reversible on recovery of Cdc7 kinase activity. Thus, newly emerging small molecule inhibitors of Cdc7 kinase have the potential to be potent and targetable therapeutic agents in the treatment of p53-mutant Her2 and TN breast cancers. Furthermore, the reversibility observed in normal cells suggests that such inhibitors would have limited toxic effects on self-renewing tissues and are therefore likely to have a broad therapeutic window.

17.00–17.15 [**PL5**] ***Diagnosis of Coronary Artery Disease Using Minimally Invasive Autopsy: Evaluation of a Novel Method of Post-Mortem Coronary CT Angiography***

Ⓟ Roberts, I[1]; Benamore, R[2]; Peebles, C[3]; Roobottom, C[4]; Traill, Z[2]

[1]Oxford Radcliffe Hospitals, Department of Cellular Pathology, United Kingdom; [2]Churchill Hospital, Oxford, United Kingdom; [3]Southampton General Hospital, United Kingdom; [4]Peninsula Radiology Academy, Plymouth, United Kingdom

There is increasing demand for a minimally invasive alternative to traditional autopsy. Cross-sectional imaging offers the greatest potential for replacing dissection but cannot assess coronary artery stenosis, the commonest cause of adult death found at autopsy. With the aim of improving diagnostic accuracy of imaging, we have developed a minimally invasive technique of coronary CT angiography that is simple, rapid and inexpensive. Radiological contrast medium is introduced into the coronary arteries by isolating the ascending aorta with a balloon catheter inserted via the left common carotid artery. We describe the first 10 cases of non-suspicious adult death in the community, investigated using this technique prior to full traditional autopsy. Imaging was reported, independent of autopsy findings, by two cardiac radiologists who described the coronary findings and provided a cause of death. The coronary arteries were visualized on imaging in all cases. Filling of the right coronary artery with contrast was limited by intravascular air in 3 cases, but an air angiogram was adequate for visualising coronary stenosis. In one case, clot in the aorta limited coronary filling but a thrombosed aneurysm of the LAD was correctly identified. The median time for performing radiology, including whole body CT and angiography,

was 41.5 minutes (27-54). There was agreement between radiology and pathology reports in 8/10 cases whilst in 2/10, angiography demonstrated significant coronary stenoses not detected by dissection. In only one of these did this result in a significant difference between the radiological and autopsy causes of death. This novel minimally invasive angiographic technique produces excellent imaging of the coronary arteries and appears to be sensitive in the detection of coronary pathology. When combined with whole body CT, the radiological cause of death correlates well with that found at full autopsy.

17.15–17.30 [**PL6**] ***The Clinical Relevance of the p53 Septin Axis and its Impact on Cellular Sensitivity to Taxol***

Ⓟ Russell, S; McKee, K; Redmond, K; Hall, P

Queen's University, Belfast, United Kingdom

Septin9 (*SEPT9*) spans 250 kb of Chr 17 encoding 18 transcripts and 15 isoforms. These isoforms exist as heterooligomers, forming cytoplasmic filaments with roles in microtubule and actin dynamics and in stress responses. The truncated SEPT9_i4 protein is encoded by two distinct transcripts. The *SEPT9_v4* transcript is regulated in a stress dependent manner by the 5' UTR and by cap-dependant mechanisms (*Hum Mol Gen* 2007;6:742-52). The *SEPT9_v4** transcript is translated by cap-independent mechanisms and is regulated in a p53 dependent manner. A *SEPT9* promoter has p53 binding sites that bind wild type but not mutant p53. This binding regulates *SEPT9_v4** but no other *SEPT9* transcript. Thus cap independent and cap dependent mechanisms can regulate the production of SEPT9_i4 protein. We investigated the biological relevance of this. SEPT9_i4 expression has effects on microtubule dynamics. Overexpression of SEPT9_i4 leads to displacement of SEPT9 protein from cytoplasmic filaments. This is paralleled by perturbed microtubule dynamics with delayed microtubule depolymerisation in response to cold shock and repolymerisation. siRNA mediated knock down of *SEPT9_v4** similarly altered microtubule dynamics. We examined the effect of *SEPT9_v4** on sensitivity to microtubule acting drugs. Compared with scrambled siRNAs, transfection of *SEPT9_v4** specific siRNA (with SEPT9_i4 knock down) leads to dramatically enhanced cell kill after taxol treatment. The clinical relevance of this is emphasised by the observation that *SEPT9_v4** expression is associated with disease stage in ovarian cancer. Our data provide novel insight into septin function and the role of stress pathways and in particular the p53 response in its regulation and has clinical significance as a determinant of drug sensitivity.

Ms K McKee was a Pathological Society PhD Student

17.30–18.30 PATHOLOGICAL SOCIETY OF GREAT BRITAIN & IRELAND'S 8th DONIACH LECTURE

Chair: Prof AH Wyllie, President, Pathological Society of Great Britain & Ireland

[**S19**] ***Back to the Future***

Prof CJL Meijer, Vrije University Medical Centre, Amsterdam, The Netherlands

For centuries it was known that cervical cancer was frequent in prostitutes and rare in nuns. So an association with an infectious agent had been postulated long ago. The finding that genital warts and cervical cancer had similar epidemiological characteristics stimulated scientists to look for the presence of "wart virus related agents" in cervical cancer and the identification of human papilloma virus (HPV) Type 16. The subsequent recognition and definition of the group of high risk HPV (hrHPV) and thourough molecular biological, epidemiological and clinico-pathological studies has led to the insight that hrHPV is the causing agent of cervical cancer .This view has stimulated scientists and clinicians to an enormous amount of basic and translational research resulting in new insights in cervical carcinogenesis and less radical ways of clinical management of precursor lesions of cervical cancer. Exiting new ways of preventing cervical cancer by prophylactic vaccination with HPV 16/18 vaccines and cervical screening by hrHPV detection in combination with molecular disease markers will change the present morphological basis of cervical cancer prevention. For the first time real control of cervical cancer in low resource countries where this disease is highly prevalent is possible. The lecture will give a personal view on this path from the past via the present to the future.

LOWER COLLEGE HALL, UNIVERSITY OF ST ANDREWS

19.30–23.00 Society Dinner

Acknowledgments (Trade Exhibition)

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Poster Abstracts

Presenter = Ⓟ

P1

Case Report: A Post Mortem Diagnosis of Pulmonary Veno-occlusive Disease

Ⓟ Ironside, A; Morgan-Rowe, L

Royal Free Hampstead NHS Trust, London, United Kingdom

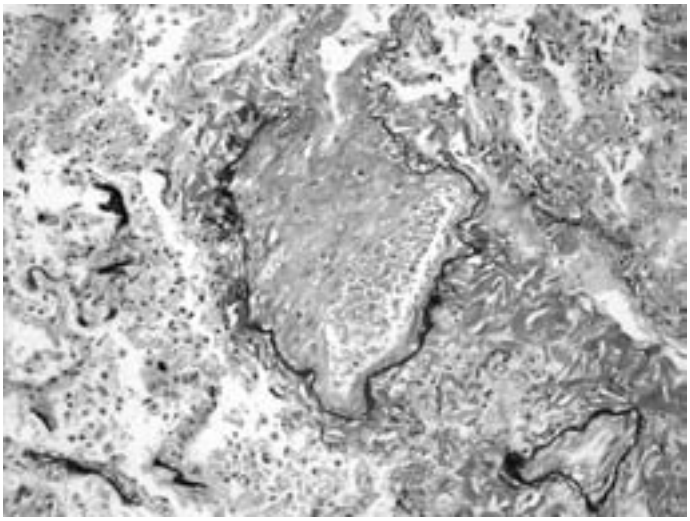
A 23 year old Albanian male presented to the Emergency Department of our institution with extreme breathlessness. He described feeling like he was drowning and was producing copious amounts of pink frothy sputum. Clinical examination revealed a patient in severe respiratory distress.

The patient continued to deteriorate and one hour after arrival was intubated, ventilated and transferred to the intensive care unit. A CT of the thorax showed peri-bronchial ground glass shadowing with consolidation and extensive mediastinal nodes. An echocardiogram showed severe pulmonary hypertension. Despite continued resuscitation efforts, he continued to deteriorate and was dead within 36 hours of admission.

This patient had previously presented to two separate accident and emergency departments with symptoms of haemoptysis, chest pain and breathlessness within the four weeks prior to this admission. A diagnosis of sarcoidosis had been suspected and the patient had been discharged on treatment. He had since failed to attend for planned follow up. It was also noted that he was a crack cocaine and heroin smoker.

Post mortem examination revealed evidence of the acute respiratory distress syndrome (ARDS) and right ventricular hypertrophy and dilatation. Post mortem histology revealed focal bronchopneumonia and significant narrowing of the pulmonary vasculature, principally affecting the small to medium sized veins with many vessels showing pinpoint stenosis. These features were highly suggestive of a diagnosis of pulmonary veno-occlusive disease (PVOD).

This case highlights that PVOD is an under-recognised and often misdiagnosed condition. Moreover, it demonstrates the value of post mortem histology in modern medicine. Firstly to provide an accurate cause of death, and secondly to highlight rare diseases such as PVOD, which may otherwise have gone completely unrecognised.



P2

Primary Mitral Valve Sarcoma Causing Sudden Death in a Child : A Case Report

Ⓟ Lawson, K[1]; Malcomson, R[2]; Sheppard, M[1]

[1]Royal Brompton Hospital, London, United Kingdom; [2]Birmingham Childrens Hospital, Birmingham, United Kingdom

Primary cardiac sarcomas are rare tumours in adult and paediatric practice. We report a case of primary mitral valve sarcoma causing sudden death in a child. A seven year old girl complained of chest pain, collapsed and died at home. At autopsy the heart was morphologically normal with a closed foramen ovale. A cord-like vegetation was adherent along part of the circumference of the mitral valve with the remainder floating free within the left ventricular outflow tract, extending through the aortic valve. Histological examination of the lesion revealed a mitotically active markedly pleomorphic spindle cell tumour with scattered multinucleate cells. The adjacent mitral valve showed involvement in the area of attachment with associated neovascular reaction. An extensive immunohistochemical panel yielded no positive results, consistent with a diagnosis of undifferentiated sarcoma. The myocardium was normal. In view of the history and autopsy examination, we find it reasonable to assume that the distal tumour occluded one of the coronary ostia leading to myocardial ischaemia and sudden arrhythmic death. This is only the sixth case of primary mitral valve sarcoma in a child, with only one previous case of sudden death due to mitral valve sarcoma (in adults or children) documented in the literature.

P3

Aberrant E-Cadherin Expression in Lobular Breast Carcinomas

Ⓟ Struthers, K; O'Donnell, M

Western General Hospital, Edinburgh, United Kingdom

Background:Invasive lobular breast carcinoma is characteristically e-cadherin negative and immunohistochemistry for e-cadherin is often used to differentiate between ductal and lobular carcinomas. However aberrant e-cadherin expression has been described in some lobular carcinomas. This may lead to some lobular carcinomas being labelled 'ductal carcinoma', which has implications for patient management and prognosis.

Aim:We aimed to identify and quantify lobular carcinomas which show aberrant expression of e-cadherin, in a cohort of cases from our institution.

Method:We reviewed 169 breast biopsies that were carried out in 2008. This included all cases that had been diagnosed as lobular breast carcinomas and all biopsies which had been stained with e-cadherin using immunohistochemistry. All the cases were reviewed by a single consultant pathologist. The H&E and e-cadherin slides were reviewed. We identified cases of carcinoma which had a lobular morphology on H&E, but which showed aberrant e-cadherin expression.

Results:Of the 169 cases, 106 showed the morphology of invasive lobular carcinoma, of the classical, pleomorphic, solid and alveolar subtypes. 26 (25%) of these cases showed aberrant e-cadherin expression. In the majority of these cases the pattern of staining was unusual, showing weak membranous staining with increased staining at the intracellular margins, compared to the strong membranous staining seen in invasive ductal carcinomas.

Discussion:These results show that e-cadherin expression in invasive breast carcinoma should not rule out the diagnosis of lobular carcinoma.

P4

Is There an Increase in Phyllodes Tumours? An Audit of Biphasic Lesions of the Breast in Edinburgh.

Ⓟ Wilkinson, I; Loane, J

Western General Hospital, Edinburgh, United Kingdom

Phyllodes tumours are rare neoplasms with a reported incidence of between 0.3 and 1% of breast tumours. On the impression of an increase in the rate of diagnosis of phyllodes tumours in our department we audited our reporting of biphasic lesions. The reports of all excised biphasic lesions from 2004 to 2008 were reviewed. From 497 operations on 474 women 545 lesions were excised (45 phyllodes (PT), 500 fibroadenomas (FA)). Of PTs 27 were benign, 17 borderline, 1 malignant. Of FAs 61 (12.2%) were reported as having atypical features (most commonly occasional mitoses and / or mild stromal cellularity). Some basic results from the audit and the rates of diagnosis per year and are tabulated:

	number	age (yrs/range)	size (mm/range)	metachronaous FA	metachronous PT
Fibroadenoma	439	34 (14-76)	23 (4-55)	34 (8.7%)	7 (1.8%)
FA with atypia	61	35 (19-55)	25.5 (9-55)	11 (18%)	2 (3.3%)
Phyllodes	45	40 (17-71)	35.4 (8-95)	n/a	1 (2.2%)

	2004	2005	2006	2007	2008
Fibroadenoma	80	84	101	64	110
FA with atypia	10	14	18	14	5
Phyllodes (%)	4 (0.6%)	7 (0.88%)	10 (1.17%)	10 (0.94%)	17 (1.87%)
Carcinoma	656	784	756	843	899

The histological criteria for diagnosis and sub-categorisation of phyllodes tumours, and for their differentiation from fibroadenomas are poorly defined. There is, in addition, an overlap between the clinical and histological features of both lesions. We report an increase in our rate of diagnosis of phyllodes tumours, suggest reasons for this and discuss the difficulties in the diagnosis and classification of biphasic lesions of the breast.

P5

An Audit of Node Positivity in Axillary Sentinel Node Biopsy/ Sampling — Would Intra-operative Assessment Improve the Patient Journey?

Ⓟ Whyte, L; Doughty, J; Wilson, C; Mallon, E

Western Infirmary, Glasgow, United Kingdom

Purpose of the study
Sentinel node biopsy is the gold standard axillary staging procedure in patients with early invasive breast cancer but lack of intra-operative assessment facilities results in many women undergoing a second operation for completion axillary clearance. Intra-operative assessment may be undertaken by frozen section or by one-step nucleic acid amplification. The aim of this audit is to determine how many cases in this unit required a second operation, demonstrating the percentage of cases that would benefit from the implementation of intra-operative cytokeratin assay analysis.
Methods
All wide local excision and mastectomy cases with a sentinel lymph node biopsy, dye-directed lymph node biopsy and 4-node sample submitted to the unit in 2009 were included in this audit. The data collected included the number of reported positive nodes, frozen section reports (if performed) and surgical outcome for node positive patients.
Summary of results
396 cases were included. A total of 52 cases (13%) with positive lymph nodes underwent a second operation for completion axillary node clearance. In addition, 50 cases had a frozen section performed at the time of initial surgery. 14 of these cases (28%) were reported positive and all these patients underwent completion axillary clearance during the initial operation.
Conclusion
In total, 66 women (17%) with node positive disease (including those with a positive frozen section) underwent completion axillary clearance. In financial terms, the guide unit cost saving in avoiding second surgery is £2,549 per patient. Therefore, the implementation of intra-operative cytokeratin analysis could remove the need for second surgery in 66 cases per year, resulting in a saving of £168,234. This saving needs to be balanced against the purchase and running costs involved in setting up the cytokeratin assay analysis in this hospital

P6

Borderline HER2 Protein Positive Breast Cancers Have Similar Patient Outcome Regardless of HER2 Gene Amplification Status

Ⓟ Barros, F; Aleskandarany, M; Rakha, E; Watts, S; Powe, D; Ellis, I; Green, A

Nottingham University Hospitals, Nottingham, United Kingdom

HER2 plays an important role in breast cancer progression and provides predictive and prognostic information. However, prognostic information provided by IHC expression categories and prognostic value added by using in-situ hybridisation (ISH) in borderline cases remains unclear. We have assessed HER2 status in a large well-characterised breast cancer series prepared as tissue microarray (n=1858) using IHC (HercepTest, DakoCytomation) and chromogenic ISH (CISH; DuoCISH, DakoCytomation) in order to identify relationships with clinico-pathological variables and patient outcome. None of these cases have received anti-HER2 therapy. There was excellent overall concordance between HercepTest negative (scores 0/1+) and positive (3+) with CISH positive/ negative (defined as HER2/Chr17 copy number ratio of ≥2; $p<0.001$). Twelve percent of cases were identified as HER2 positive (those with 3+ HercepTest scores or 2+ with gene amplification). Of the 74 borderline HercepTest 2+ cases, 44 cases (59%) showed HER2 gene amplification. We identified that HercepTest 2+ non-amplified cases were not significantly different from those amplified 2+ or 3+ cases with respect to their clinical outcome (BCSS and DFS). The overall concordance between HercepTest and CISH analysis for HER2 status was excellent. All HercepTest 2+ cases identified were observed to have poor outcomes similar to those HercepTest 3+ cases regardless of gene amplification status. In the current clinical environment, cases exhibiting IHC 2+ with non-amplified HER2 gene status are not offered targeted HER2 therapy but do exhibit aggressive clinical behavioural characteristics and therefore optimal treatment strategies for these patients need to be determined.

P7

The Histone H4 K16 Regulatory Axis in Breast Tumours: Modulation by Small Molecule Inhibitors

Ⓟ Abdelghany, M[1]; Collins, H[1]; Green, A[2]; Ellis, I[2]; Heery, D[1]

[1]Gene Regulation Group, School of Pharmacy, University of Nottingham, Nottingham, United Kingdom; [2]School of Molecular Medical Sciences, University of Nottingham and Nottingham Universities Hospital Trust, Nottingham, United Kingdom

Breast tumours display significant phenotypic and molecular heterogeneity. Recent research has attempted to generate improved molecular classifications of breast tumours using gene expression profiling, and tumour microarray immunohistochemistry. We recently reported that global levels of posttranslational modifications (PTMs) in core histones are radically altered in breast tumours, and that these factors show a strong association with tumour phenotype, prognostic factors and patient survival (El-Sheikh et al, 2009). In this study, we focussed on a key histone PTM (acetyl H4K16), and profiled the expression of a range of chromatin modifying enzymes that modulate H4K16 and other histone PTMs in 880 primary breast carcinomas. Our data show significant associations of these PTMs with the expression of HAT and HDAC factors and their regulators. In addition, using a MCF7 model, we show that small molecule inhibitors such as garcinol can modulate the expression levels of chromatin modifiers, and reprogram cancer-associated PTMs, including AcH4K16. This is accompanied by inhibition of proliferation through a blockade of S phase and cell cycle arrest.

P8

Expression of the Trefoil Protein TFF3 in Human Breast Cancers

Ⓟ Ahmed, A[1]; May, F[2]; Tilby, M[2]

[1]Faculty of Medicine, Sohag, Egypt; [2]Northern Institute for Cancer Research, Newcastle Upon Tyne, United Kingdom

Trefoil factors are small proteins that are secreted from mucus secreting epithelia. Motogenic actions of trefoil proteins may help tumour cell invasion and metastasis. TFF3 mRNA expression is regulated by oestrogen in breast cancer cell lines. Association between oestrogen receptor and TFF3 mRNA expression has been demonstrated in breast tumours. TFF3 protein has not been analysed previously in human breast cancers. A tissue microarray was constructed from 300 primary breast tumours and 76 metastatic deposits. TFF3 expression was evaluated by immunohistochemistry with an antibody raised against correctly-folded human TFF3. The intensity of immunoreaction in 1,000 tumour cells was evaluated as: absent, weak, moderate or strong. The association of TFF3 expression with clinico-pathological features and with oestrogen receptor expression was tested statistically with SPSS software ($p<0.01$). TFF3 is expressed in normal and malignant breast epithelial cells and not expressed in stromal, endothelial or immune cells. Expression of TFF3 varied enormously between tumours; 238 cases were positive. TFF3 expression is highest in mucinous and tubular breast carcinomas. It is expressed at higher levels in lobular than in ductal cancers. TFF3 expression is associated strongly with oestrogen and progesterone receptor expression. There is a negative association between TFF3 expression and tumour grade. There is however a positive association between TFF3 expression and presence of vascular invasion and presence of axillary LN metastasis. TFF3 expression is higher in metastatic breast tumour cells than in primary breast tumour cells. The association between TFF3 and oestrogen receptor suggests that TFF3 expression is dependent on oestrogen in breast tumours. The high expression of TFF3 in breast tumours with vascular invasion and in metastatic tumour cells supports the hypothesis that TFF3 predisposes towards breast cancer cell invasion.

P9

A Falsely Positive (C5) FNAc from a Lymph Node with Benign Vascular Transformation of the Sinuses (VTLNS)

Ⓟ Grigor, T; Jones, H

Royal Cornwall Hospital, Truro, United Kingdom

A 70 year old woman presented with a symptomatic breast lump while part of the breast screening programme. Mammography demonstrated a calcified mass, 30mm diameter and a 9mm, radiologically indeterminate, ipsilateral axillary lymph node. Ultrasonography of the symptomatic mass was sonographically malignant (U5). The axillary tail lymph node had an sonographically indeterminate echogenic centre. Ultrasound guided needle core biopsy showed Grade 2 IDC. The cytology smear from the axillary lymph node was reported as falsely positive for carcinoma cells (C5). A right mastectomy and axillary node clearance was performed. Histological examination demonstrated a Grade 3 IDC with high grade comedo ductal carcinoma in situ. Pathological node status of the specimen was ascertained from 19 lymph nodes in the tail of the mastectomy specimen (level I nodes), nine in a separate piece of tissue incorporating level II nodes (largest 13mm) and 4 level III nodes in a piece of apical tissue. All 32 lymph nodes examined were free of tumour (pT2, pN0, pMx). However several showed characteristic VTLNS with an intra-sinusoidal proliferation of endothelial cells stainable for VWF accompanied by an intra-sinusoidal fibrous reaction. Pre-operative staging of the axilla using FNAc can triage women with operable breast cancer prior to an initial nodal surgical procedure. VTLNS is an example of a benign process that can simulate metastatic involvement of a lymph node by carcinoma diminishing the accuracy of this test.

P10

Hypersensitivity Pneumonitis-like Changes in Patients Treated for Haematological Malignancy–in an Abnormal Immunologic State What Disease Process Does it Reflect and is it Under Diagnosed?

Ⓟ Fraser, S[1]; Pomplun, S[2]

[1]Preston Hall Hospital, Maidstone, United Kingdom; [2]King's College Hospital, London, United Kingdom

The development of pulmonary complications is common in patients with haematological malignancy and is associated with significant morbidity. The main differential diagnoses for clinically significant lung abnormalities include infections and drug reactions. As this differential diagnosis is important in determining patient management open lung biopsies are being performed in cases with equivocal radiological findings.
We reviewed all open lung biopsies performed on haematology patients over a one year period. The predominant histological findings were those of hypersensitivity-like pneumonitis rather than obvious viral or fungal infections. This may reflect patient preselection however it is unclear whether this pattern of lung infiltration represents an incomplete immune response to an infective trigger or whether it reflects true drug/hypersensitivity reactions which are under diagnosed in altered immunologic states and might occur more commonly than previously thought.

P11

Sudden Cardiac Death (SCD) in Individuals with a History of Alcohol Use with or without Antipsychotic Medication and/or Class A-C Drugs.

Ⓟ Alani, H[1]; de Noronha, S[1]; Patel, J[1]; Sheppard, M[2]

[1]CRY Centre for Cardiac Pathology, Imperial College, London, United Kingdom; [2]CRY Centre for Cardiac Pathology and Royal Brompton & Harefield NHS Foundation Trust, London, United Kingdom

Alcohol-related deaths in the UK have steadily increased, rising from 4,023 in 1990 to 9,021 in 2008. Most are non-cardiac. This study aims to highlight cardiac causes particularly within the younger generation. One hundred and sixty-five cases of SCD with a history of alcohol were referred to our specialist cardiac pathology centre from January 1996 to February 2010. Drinking patterns were categorised into 4 groups: alcohol prior to death (n=100), binge drinker (n=32), chronic alcoholism (n=54, of which almost half had fatty liver/cirrhosis and 8 had alcohol withdrawal) and moderate to heavy drinker that could not be classified into binge or chronic (n=21). The majority of cases were young males (n=114, 69%), mean age 35±12.8 years, range 15-76 years. Adolescents (<20 years) made up 10% and 40% were ≤30 years of age. Some also took class A-C drugs (n=47) and/or had mental health problems taking antipsychotic medication (n=24). An important finding is that half died suddenly with a morphologically normal heart at both macroscopic and microscopic level (n=82), strongly suggesting the possibility of channelopathies, e.g. Brugada and long/short QT. Additionally, these SCD may have occurred through a fatal arrhythmia precipitated by alcohol use. Cardiomyopathy was also a dominant cause of death (n=49) followed by coronary artery pathology (n=16). Other important causes were toxic myocarditis (n=11), CHD (n=5), aortic dissection (n=1) and an AV nodal tumour (n=1). This study highlights the importance of SCD linked to a history of alcohol use. Our study also raises awareness of SCD in individuals that had consumed non-toxic levels of alcohol just prior to their death and emphasizes the risk of the pro-arrhythmic effects of alcohol in those who have underlying cardiac conditions.

P12

Cytospin Preparations Provide Better Quality Diagnoses Than Direct Smears in Nodal Needle Aspiration Specimens For Non-Small Cell Carcinoma (NSCLC): A Multicentre Review.

Ⓟ Rowan, C[1]; Tournoy, K[2]; Dooms, C[3]; Rintoul, R[4]; Annema, J[5]; Shah, P[1]; Rassl, D[4]; Nicholson, A[1]

[1]Royal Brompton Hospital, London, United Kingdom; [2]Ghent University Hospital, Ghent, Belgium; [3]Leuven University Hospital, Leuven, Belgium; [4]Papworth Hospital, Papworth, United Kingdom; [5]Leiden University Medical Center, Leiden, Netherlands

Background: Diagnostic cytology is undertaken mainly via direct smear (DS) or cytospin (CS) preparations, with usage often dependent on institute and operator preference. The ASTER trial, a prospective randomized trial to compare immediate surgical staging (SS) against endosonography (ES) followed by (SS), was a multicentre (n=4) project that provided an opportunity to prospectively compare the two techniques.
Methods: 119 cases from the ES/SS arm, prepared and diagnosed locally, two using DS (mainly May-Grunwald-Giemsa stained) (n=83) and two CS (mainly Papanicolaou-stained) (n=36) preparations, were subsequently reviewed by a reference pathologist, with number of slides per case/nodal station, % positive cases/stations, time per case, and interobserver (IO) agreement recorded. A further comparison was made from the laboratory of the reference pathologist where practice changed from direct smear (n=52) to cytospin (n=55) in 2006.
Results: For CS versus DS, there was 100% versus 97.5% IO agreement, taking 12.5 versus 27.2 mins/case (av. 4.7/1.3 versus 14.8/5.6 slides per case/station), but with lower positive pick-up rate per case/station (38.5/17.9 versus 60/29.3% %). However, the non-ASTER data set from a single institution (CS vs DS) showed a higher positive pick-up rate with CS than DS (56.4/46.2 vs 38.5/34.9% (case/station); slides per case/station 3.53/2.13 vs 8.38/5.25; inadequacy/poor quality rate (4.6% vs 23.1%)).
Conclusions: CS allows quicker diagnosis (less slides/better quality preparations) and has better IO agreement. CS also allows cell pellet preparation for further refinement of diagnosis of NSCLC. The lower positive pick-up rate in the ASTER trial was reversed in the single institution experience, likely reflecting variation in institution practices and patient selection.

P13

Out of Hospital Fatal Myocardial Infarction

Gallagher, P; Ⓟ Joughin, D; Lockyer, B

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In hospital mortality from acute myocardial infarction has decreased progressively for more than 20 years. In some centres it is now as low as 6%. In contrast there is only limited evidence that the number of sudden cardiac deaths in the community is decreasing. Historic studies from the 1990s using extensive histology indicated that about 50% of community sudden cardiac deaths were the result of acute coronary thrombosis or myocardial infarction. There is a clinical suspicion that the incidence of ST elevation myocardial infarction is decreasing. We therefore studied the proportion of cases of sudden cardiac death that were the result of acute coronary events. Our hypothesis was that these would be decreased. We made a prospective study of 114 sudden deaths. 45% were non cardiac, chiefly pulmonary emboli, ruptured aneurysms or due to lung disease. 40.3% of cardiac deaths were the result of acute myocardial infarction or coronary thrombosis. 38.7% were due to coronary artery narrowing with or without healed myocardial infarction. 20. 9% were associated with heart failure or ventricular hypertrophy. In a similar audit performed in 2002-3 we found that 37% of more than 366 sudden cardiac deaths were the result of acute coronary events. Although this is a much smaller study it does not suggest that the incidence of out of hospital myocardial infarction is decreasing. It provides further support for the rapid transfer of patients with chest pain to cardiac centres.

P14

Do Papillary Carcinomas Arise in Thyroglossal Cysts?

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A 68 year old male presented with a longstanding, non-tender, midline swelling of his neck that appeared to move on swallowing. Serum biochemical analysis was unremarkable (TSH 1.15 mIU/L). Pre-operative imaging or cytological assessment were not obtained. An excision biopsy was performed. Macroscopically the specimen comprised of scanty fatty tissue surrounding a smooth, thin-walled 16mm diameter cyst with a smooth inner lining containing a small amount of straw coloured fluid. Light microscopic examination showed a typical thyroglossal duct cyst architecture. Focally there were intra-luminal papillary projections covered by CK-19 and thyroglobulin positive cells that had overlapping, occasionally grooved nuclei, without the typical 'Orphan Annie' appearance. Intracystic aggregates of pigmented macrophages admixed with reactive fibrosis and a lymphocytic inflammatory infiltrate were also a feature. There were two lymph nodes in the wall of the cyst; one appeared to contain crushed epithelial cells in a sub-sinus location that were stainable for thyroglobulin antibody. Specialist review confirmed a diagnosis of Cystic Papillary Carcinoma arising in thyroglossal duct remnants with a micrometastasis to a lymph node adjacent to it. This patient's case is interesting because of its rare and unusual mode of clinical presentation and the necessary exclusion of benign papillary hyperplasia as a possible alternate diagnosis. Specific light microscopic features, immunohistochemistry and the fortuitous inclusion of an adjacent 2mm lymph node containing a micrometastasis were the keys to this case. Kusunoki et al. Thyroid. 2007 Jun; Vol 17(6):591-2. *Carcinoma arising in thyroglossal duct remnants.*

P15

Primary Leiomyosarcoma in the Parotid, Tongue and Foot of Three Adults

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Background: Leiomyosarcoma is an uncommon malignant tumour with smooth muscle differentiation that often occur in the soft tissue of the gastrointestinal tract, reproductive organs and retroperitoneum. The tumour may however be seen in any anatomic site where smoth muscle is found. Cases : We describe three adults ; a 28year old female student and two males aged 35years and 56years with leiomyosarcoma of the dorsum of foot, left parotid and tongue respectively. The female presented with a year history of swelling of the dorsum of the left foot, the younger man presented with two months history of ulcerated discharging left facial swelling and the elder man with a tongue nodule. Histology of tissue biopsies from the three lesions showed a malignant infiltrative tumours growing in diffuse and haphazard fascicles. They were composed of pleomorphic spindle cells having hyperchromatic to vesicular cigar shaped blunt ended nuclei, prominent 1-3 nucleoli with fibrillary cytoplasm. Tumour giant cells and mitotic figures of over 6-15 per 10HPF were seen within a fibrocollagenized stroma. Conclusion: Leiomyosarcoma of soft tissue is relatively rare and constitute 6.5% of all soft tissue sarcoma. It is an aggressive tumour with propensity for the extremity and less than fifty cases have been reported in these three sites combined. Diagnosis is based on hematoxylin- & eosin-stained histologic sections with or without special stains. Other diagnostic techniques include electron microscopy, immunohistochemistry and cytogenetics for increased accuracy and differentiation from other soft tissue sarcomas

P16

The Diagnosis of Head and Neck Cancer Using Fine Needle Aspiration Cytology

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Purpose of Study: Head and Neck (H&N) cancer in the UK accounts for around 8000 cases/year. Fine Needle Aspiration Cytology (FNAC) is recommended as a 1st line investigation in palpable H&N masses, salivary gland and thyroid lumps. However, a particularly high level of expertise is required to achieve a precise and reliable diagnosis. This study aimed to assess the accuracy and value of cytology in diagnosis when compared to histopathology. Methods: 260 FNACs were reported at Bradford Hospitals from 2/2007 to 2/2008, of which 120 had subsequent histological assessment, 44 were insufficient and the remainder had no documented follow-up. All cyto- and histo-pathology was critically appraised for diagnostic testing (included analysis of positive/negative predictive value (PPV/NPV), sensitivity and specificity). Summary of Results: Analysis of the 120 cases returned a concordance of 80.83% (within each sub-category neck-nodes 76%; neck-lumps 95%, parotid/salivary glands 87.5% and thyroid lumps 78%). Importantly, 17% of FNACs were considered insufficient and the diagnosis relied on histopathology. Overall, analysis showed that FNAC had a PPV=93% (95% CI 0.86-0.86), Sensitivity=86.95%, Pre-test probability (prevalence)=84.25%, NPV=48.15% (95% CI 0.31-0.66), Specificity=65%, Accuracy=83.46%. Conclusions: A recent meta-analysis has shown FNAC to be a highly effective in the diagnosis of H&N cancers with some limitations. Our results showed that FNAC had a relatively poor NPV (the proportion of patients with negative test who are correctly diagnosed) = 48.15%. Also, we found FNAC to be a sensitive but not specific technique. The accuracy of FNAC is related to aspirator skill and the experience of the cytopathologist. FNAC has reduced the number of patients requiring surgery by 35-75%. Our analysis returned a NPV=48% and FNAC insufficiency rate=17%. Image-guided FNAC may improve sample quality and potentially NPV and specificity

P17

The Role of Postoperative Radiotherapy in the Management of Parotid Pleomorphic Salivary Adenomas: Is There Any Benefit?

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Introduction Pleomorphic Salivary Adenoma is the most common tumour of the Parotid Gland. Currently no national management guidelines exist. The objective of this study was to evaluate the role of adjuvant radiotherapy. Methods A retrospective study of all patients with a histological diagnosis of PSA between 1981 and 2008 in Greater Glasgow and Clyde was undertaken. From intra- operative notes and pathology reports, adherence to facial nerve, excision margins, capsule status and postoperative radiotherapy were analysed. Two cohort groups were identified. The first cohort underwent surgery alone while the second received postoperative radiotherapy. Post-operative recurrence, short and long- term complications were compared in the two groups. Results 201 patients were identified. 167 (83%) had surgery alone and 34(17%) received adjuvant radiotherapy. Medical notes were retrievable in all patients receiving postoperative radiotherapy and in only 58 surgical patients .The rate of recurrence was 1.7% (1/58) in surgical patients and 2.9% (1/ 34) in patients receiving adjuvant radiotherapy . Short -term complications were significantly higher in the second cohort accounting for 100% compared to 38% in the first. While long- term complications 15/58 (25%) and 12/34 (32%) were observed in the first and second cohort respectively. Conclusions There was no significant difference in the recurrence rate between the two groups. Short term and long term complications were significantly higher in the postoperative radiotherapy cohort. Adjuvant radiotherapy is therefore not recommended in the treatment of PSA. As well as a higher long term complication rate, radiotherapy is less cost effective.

P18

Bone Marrow Fibrin Ring Granulomas in Epstein Barr virus infection

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Fibrin ring granulomas are composed of a central lipid-filled vacuole surrounded by a ring of fibrin and epithelioid macrophages. They are seen in the bone marrow and liver in association with a variety of aetiologies, including infective agents, T cell lymphomas and allopurinol hypersensitivity. Infective aetiologies include Coxiella burnetii (Q fever), cytomegalovirus, hepatitis A and, in isolated case reports in the English literature, Epstein Barr Virus (EBV). We present a 36 year-old Caucasian female who presented with pyrexia of unknown origin, generalised lymphadenopathy, abnormal LFTs and raised LDH. A monospot test for glandular fever at presentation was negative, and bone marrow trephine biopsy and cervical lymph node excision were carried out. Fibrin ring granulomas were present in the bone marrow trephine whilst the lymph node showed features strongly suggestive of EBV infection, including positive in situ hybridisation (EBERs) and immunohistochemistry (LMP-1 and EBNA-2). A repeat monospot test was positive and the patient's blood raised titres of EBV IgM and IgA, suggesting recent acute infection. Tests for CMV and hepatitis C were negative. The patient made an unremarkable recovery and was discharged shortly following diagnosis. Fibrin ring granulomas are rarely encountered in pathological specimens, but their presence should stimulate an active workup of the patient for infectious agents, including EBV.

P19

Unrelated Clones and Acquired BCL2 Gene Abnormalities in Cutaneous Marginal Zone Lymphoma

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Lymphomas are believed to derive from a single transformed lymphoid cell. Translocations, and much less frequently amplifications, of the BCL2 gene are the initiating event in most follicular lymphomas(FL), the former occurring in the bone marrow during immunoglobulin gene rearrangement. The t(14;18)(q32;q21) involving the BCL2 gene has also rarely been reported in extranodal marginal zone lymphoma (MZL). Herein we document two unique cases of cutaneous MZL associated with BCL2 gene abnormalities.

Both patients presented with typical features of MZL; neoplastic infiltrates of small lymphocytes, numerous light chain restricted plasma cells and residual reactive follicles. One patient suffered 6 relapses with a common clone demonstrable by PCR in the 1st, 3rd and 4th, but an apparently unrelated clone in the 2nd. In addition, a previously undetected BCL2 gene translocation was found in the 5th and 6th relapses by interphase FISH. This correlated morphologically with a collection of neoplastic follicles in an otherwise typical MZL in relapse 5, whilst the pathological features in relapse 6 were of a typical FL. The other patient relapsed 3 times, genetically unrelated abnormal clones being demonstrated by karyotyping on each occasion. Marked amplification of the BCL2 gene was also identified by FISH in the third relapse but in none of the earlier biopsies. This coincided with acquisition of CD10 and BCL6 by a proportion of the neoplastic lymphocytes.

These two cases illustrate that abnormalities of the BCL2 gene may occur in cutaneous MZL. They occurred as secondary events, and unlike most previous cases of MZL with BCL2 gene abnormalities, coincided with an alteration in phenotype to one more closely resembling FL than MZL. Although not proven, the genetic findings also raise the possibility that relapses in some cutaneous MZL represent separate clonal events rather than recurrence of the original abnormal clone.

P20

Review of EBV-Positive Diffuse Large B-Cell Lymphoma Occurring in Immunocompetent Patients

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Epstein-Barr virus (EBV) positive diffuse large B-cell lymphoma (DLBCL) of the elderly has recently been included in the WHO classification of malignant lymphoma. It usually occurs in the elderly with no underlying immunodeficiency and is associated with a poor prognosis. We have undertaken a review of EBV-positive DLBCL occurring in immunocompetent patients in the West of Scotland in order to define its clinical and pathological characteristics. All cases of EBV-positive DLBCL in patients with no known history of immunosuppression reported at Glasgow Royal Infirmary from 2001 to 2009 were reviewed. The clinical, morphological and immunophenotypic features were recorded. 26 patients were identified with a median age of 66 (range 18 to 97) and a male female ratio of 1:2, representing approximately 7% of all DLBCL. There were 11 nodal and 15 extranodal presentations with 7 occurring in the nasopharynx or oral cavity. Three morphological patterns were identified. A typical DLBCL pattern (n=9), a plasmablastic (n=6) and a Hodgkin-like pattern (n=11). 17 cases in which full immunophenotyping was performed had an activated B-cell immunophenotype (MUM1+ CD10 neg). CD30 was expressed in 88%. 58% of patients presented in stage 3 or 4 (slightly higher than the regional figure of 51% for all DLBCL). 7 patients died, 3 within the first month following diagnosis. The majority of those fit enough for chemotherapy achieved complete remission. EBV positive DLBCL in patients not apparently immunosuppressed occurs over a wide age range though is most common in the elderly. Extranodal presentations in the upper aerodigestive tract are particularly common. In cases of DLBCL, plasmablastic or Hodgkin-like morphology, activated B cell phenotype and CD30 positivity should trigger testing for EBV by ISH. The prognostic implication of EBV-positivity in this small cohort is unclear at the time of reporting.

P21

A Role of Small-Sized Cell Population in Tumourigenesis of Hodgkin Lymphoma Cells

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Tumours consist of heterogenous cell populations derived from a single clone. Recently, it has been demonstrated that cells with tumourigenic potential are limited to a small population, called cancer-initiating cells or cancer stem cells (CSCs), in several tumours, such as leukemia, breast, brain, and colon cancers. To date, such a population has not been identified in malignant lymphomas. Here, we examined the presence of CSCs in Hodgkin lymphoma (HL). HL cell line L1236 consists of heterogeneous sized cells; single-nucleated small-sized cells (S cells) like Hodgkin cells and multinucleated large-sized cells (M cells) like Reed-Sternberg cells. To examine the difference of tumourigenicity between S and M cells in L1236 cells, limiting dilution, semi-solid cultures, and injection into non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice were performed. In limiting dilution, S cells showed a more proliferative potential than M cells. S cells yielded both S and M cells. In contrast, M cells with less proliferative potential, yielded only M cells. In semi-solid cultures, S cells formed colonies more efficiently than M cells. In contrast to S cells, proliferative potential of M cells was rarely observed in limiting dilution assay. At injection into NOD/SCID mice, S cells formed tumours more efficiently than M cells. These results suggested that S cells were more tumourigenic than M cells. CSCs are known to contain a lower level of reactive oxygen species (ROS) than non-CSCs. A part of S cells contained lower levels of ROS than M cells. ROS-low cells are known to express FoxO3a, which enhances the expression of ROS-excluding enzymes, such as superoxide dismutase and catalase. FoxO3a was expressed in a part of S cells, suggesting that the FoxO3a-expressing cells might be a candidate for CSCs. These findings suggest that S cells might play an important role in tumourigenesis of HL.

P22

A Review of Lymphoma in Non-transplant Patients on Immunosuppressive Therapy

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Iatrogenic immunodeficiency-associated lymphoma in non-transplant patients, now a specific category in the WHO classification of malignant lymphoma, is important to recognise as regression may occur following immunosuppression (IS) withdrawal. As there are many questions regarding management of these cases, we have undertaken a review of our experience in the West of Scotland.

We identified 45 cases of lymphoma developing in patients treated with Methotrexate (40) or Azathioprine (5) from pathology and rheumatology databases over a 10 year period. 33 patients suffered from rheumatoid arthritis, 4 from psoriasis and the remainder from other conditions. There were 20 cases of Diffuse Large B Cell Lymphoma (DLBCL), 12 of classical Hodgkin Lymphoma (cHL), 2 of Hodgkin-like lymphoma, and 11 of other lymphoma subtypes including Follicular Lymphoma and T-cell Lymphoma.

Where EBV status was known, 55% of all cases, 61% of DLBCL, 75% of cHL and 100% of Hodgkin-like Lymphomas were positive. EBV-positive DLBCL had an activated B cell immunophenotype in 82% of cases.

Two patients are in remission as a result of IS withdrawal and 7 patients (2 DLBCL, 4 cHL and 1 Hodgkin-like) developed a transient response to IS withdrawal. One of the latter had a stable response with the addition of Rituximab. The remainder required chemotherapy or radiotherapy. Eight of the 9 cases responding to IS reduction were EBV positive.

A wide variety of lymphoma subtypes occur in patients receiving immunosuppression for inflammatory diseases. Though most will eventually require chemo/radiotherapy, occasional cases will develop complete remission with IS withdrawal alone or in combination with Rituximab. A trial of immunosuppression reduction is worthwhile in all cases, independent of EBV status.

P23

The Relationship of Bone Marrow Trephine Length, Adequacy and Disease Process

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Bone marrow trephine biopsies are essential in the diagnosis and staging of haematological and non-haematological diseases. Absolute clarification of what constitutes adequacy is lacking, with 15mm having been suggested as the minimum adequate length. Ultimately, however, adequacy must be defined by the ability of a reporting pathologist to produce a definitive diagnosis from the trephine, accepting boundaries of good practice. We examine three increasingly specific bone marrow data-sets to determine a pragmatic guide for adequacy that is useful for clinicians and can help pathologists. Our data-sets comprise: (i) a non-selected "base-line" set, comprising 100 sequentially received cases; (ii) a set comprising 700 consecutive trephines taken as staging for previously diagnosed lymphomas of any type and (iii) a "focal" sub-set comprising 268 staging trephines for lymphomas associated with focal marrow involvement. We use a novel application of logistic regression to model the probability of a biopsy being regarded as adequate. For our base-line set, a length of 15.7mm is required in order to obtain a 95% probability of a specimen being regarded as adequate in our practice. For lymphomas in general, 95% probability is reached with a length of 17.2mm, whilst for our focal set 21.5mm is required. The average length in all our sets is less than optimum by these standards. We also find that, when trephine length is short, each 1mm more can make it up to 30% more likely to produce a diagnostic report. In conclusion, the likely adequacy of a bone marrow trephine relates to the disease being investigated as well as the absolute length of the specimen. Thus, the indication should be taken into consideration when assessing adequacy. To ensure the procedure serves its purpose, obtaining a longer biopsy in staging focal lymphomas would ensure more accurate results with prognostic benefits.

P24

Plasma Cell Myeloma Presenting as a Testicular Mass: A Case Report

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Introduction: This is a case report of plasma cell myeloma presenting as a testicular mass in an otherwise fit 58 year old man. Case: A 58 year old man presented to his general practice with a 2 month history of unilateral, painless testicular swelling. He did not complain of weight loss, back pain or bone pain and was otherwise fit and healthy. Clinical examination revealed a large testicular swelling, non-tender, which clinically resembled a hydrocele. Ultrasound examination performed shortly after showed a large tumour mass and urgent orchidectomy was performed. Tumour markers, alpha-fetoprotein and beta HCG were normal. Pathological assesment revealed a grossly enlarged 110cm testicle. The cut surface appeared lobulated and showed a white homogenous tumour replacing the entire testicle. Microscopically, the tumour was made up of numerous sheets of large lymphoid blast cells mosty resembling immunoblasts. Initial immunohistochemistry showed strong expression for MUM-1, patchy CD79a positivity and negative staining for CD20, CD10, BCL-2 and BCL-6. Further immunohistochemistry with plasma cell markers CD138 and CD56 showed strong positivity. CD 45 however was negative. A diagnosis of plasmacytoma or possible deposit of plasma cell myeloma was suggested which prompted further investigation and haematological referral. Subsequent investigations including laboratory tests and serum electrophoresis which were supportive of the diagnsosis. The bone marrow trephine performed confirmed the presence of plasma cell myeloma. Discussion: Plasma cell myelomas charecteristically form multiple tumours involving the skeletal system multifocally. Patients can have extramedullay disease and common sites include the liver, spleen and lymph nodes. Localisation to the testis is considered rare, particularly as an initial presentation.

P25

Paratrabecular Distribution of Lymphoplasmacytic Lymphoma in Bone Marrow

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Lymphoplasmacytic lymphoma (LPL) is a rare neoplasm composed of small B lymphocytes, plasmacytoid lymphocytes and plasma cells that usually involves the bone marrow. Marrow involvement may be nodular, diffuse and/or interstitial and can also show paratrabecular aggregates. Published data suggests that one-third of LPL cases show a paratrabecular component. This study looked at a large series of LPL to determine the proportion showing paratrabecular disease and to quantify the extent of the paratrabecular component. A search over the last 5 years yielded 126 bone marrows with a diagnosis of LPL. All cases with a CD20 and/or Pax5 were reviewed (106 cases) and percentage involvement by LPL and percentage paratrabecularity were assessed. Cases showing no evidence of disease (10 cases), where there was >75% involvement, and/or where the specimen was <5mm in length were excluded. Of the 69 remaining cases, 32% showed 50-75% overall involvement by LPL, 26% showed 25-50% involvement, and 42% showed <25% involvement. A paratrabecular component (>10%) was seen in 62% of cases. Further quantifying this component showed the following; no cases were >90% paratrabecular, 19% of cases showed 50-90% paratrabecular disease, and 43% of cases were 10-50% paratrabecular. 38% of cases were <10% (i.e. not) paratrabecular. Our study indicates that the proportion of lymphoplasmacytic lymphomas in bone marrow with a paratrabecular component is higher than that previously published, and that this pattern may be a useful diagnostic feature in LPL.

P26

Audit of Central Review Cases to Identify Trends in Light of the NICE IOG on Haematological Cancers

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A review of all lymphoma cases received, during a period of just under one year, for central review at a specialist referral centre in one cancer network was undertaken. 300 cases were reviewed. Out of these the original report was not available in 41 cases. Of the remaining 259 cases, the original (referring pathologist's) diagnosis was agreed with in 196 cases. There was discordance between the referring pathologist's diagnosis and the diagnosis after specialist review in 63 cases. The discordant diagnoses were subclassified into categories 1-3 based on the Royal College of Pathologists guidelines on categorisation of discrepancies in histopathology (2008). Of the 63 cases, 26 were found to be category 1 discrepancies which included a diffuse large B-cell lymphoma being diagnosed a Seminoma, Nodular Lymphocyte Predominant Hodgkin Disease being called classical Hodgkin Lymphoma and no report on transformation to diffuse large B-cell lymphoma in follicular lymphoma, amongst others, which were known to have therapeutic and clinical ramifications. Also, 25 cases were found to be category 2 discrepancies which included wrong subclassification of B-cell lymphomas (e.g. lymphoplasmacytic lymphoma being called marginal zone lymphoma). 12 cases were found to be category 1 of which the commonest reason was grading of follicular lymphoma and classical Hodgkin lymphoma. Although the last category includes criterion which are subjective, in any cancer centre which undertakes research, accurate subclassification is important. This highlights the importance of specialist referral in haematopathology as per NICE guidance for both diagnostic and research work.

P27

Is Endoglin a Particular Marker for Endothelial Cells in Glioblastoma Multiforme?

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Abstract background: Angiogenesis is an important factor in the growth of solid tumours that can be used in their diagnosis and treatment. Aim:To compare CD31 and CD105(Endoglin) staining in Glioblastoma multiforme angiogenesis. Methods:This study was performed on 50 Glioblastoma multiforme(GBM) samples, reffered to Al-Zahra hospital pathology lab between 2001 to 2006. We prepared 3 slides from each sample which were used for immunohistochemistry for CD31, CD105(Endoglin), and Ki67(proliferation Index) monoclonal antibodies. Microvessel density(MVD)was evaluated by immunostaining for CD31 and CD105 and the results were compared between the two and also with Ki67 expression. Results:We showed that CD105-MVD was significantly higher in Glioblastoma compared with normal tissue(14.28 vs. 6.68: P=0.012). We did not find such difference for CD31. The mean of CD105-MVD was significantly higher than CD31-MVD in Glioblastoma tissue(P<0.001) although there was a significant positive relationship between them(Pearson's r=0.630 P<0.001). CD105 odds ratio was 11:1. 95% confidence interval: 3.54-36.53. The mean of proliferation index was more closely correlated with CD105-MVD(Pearson's r=0.611 P<0.001) than CD31-MVD(Pearson's r=0.360 P=0.01). Conclusion:We suggest that Endoglin can be used as a specific and sensitive marker for evaluation of angiogenesis in Glioblastoma which can be used in the diagnosis and treatment of this tumour. Comparing these results with previous studies revealed the importance of Endoglin in the prognosis of Glioblastoma.
Keywords:CD105,CD31,Angiogenesis,Glioblastoma Multiforme (GBM),Vascular Endothelial Growth Factor (VEGF)

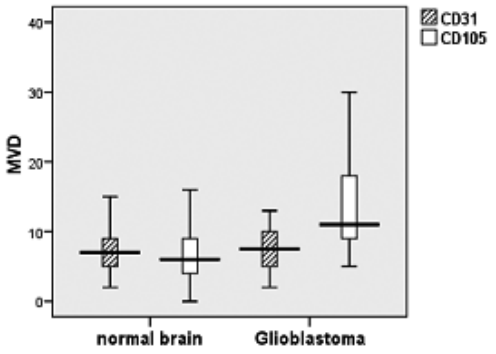

Figure 1: Mean of CD105 microvascular density (MVD) and CD31-MVD in glioblastoma and normal brain tissue.
t-student P=0.807 for CD31-MVD
t-student P=0.012 for CD105-MVD

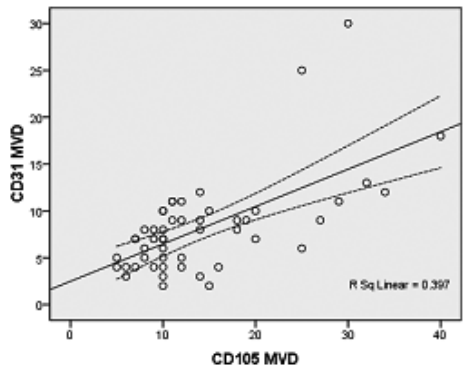

Figure 2. Correlation between CD105 microvessel density (MVD) and CD31-MVD in glioblastomas (Pearson's r=0.630 P<0.001).

P28

A Case Report of Alkaptonuric Ochronosis with Monoarthritis

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A 47 year old male admitted with post operative infection in right knee. He had a history of pain and swelling(arthritis) in right knee from 2 months ago and a loose body and DJD changes was seen in X-ray studies. Knee joint biopsy was reported as synovial osteochondromatosis and then he underwent surgery. Because of continuous infectious secretions and failure in treatment he was referred to our medical center. Past history : he suffered from Hyperthyroidism and after radiotherapy it changed into Hypothyroidism and uses daily Levothyroxin. He had a mild and insidious low back pain from several years ago.
Physical examination : small bluish pigmentation was seen on thenar area of hands which was diagnosed as AVM malformation.
Roentgenography: dorsolumbar spine x-ray revealed decrease in intervertebral disk spaces and fusion of 2 spines with calcification. Osteoarthritis changes were also seen.
Lab data : all routine laboratory findings like CBC/diff, FBS, Ca/P,U/A, ANA were in normal limits.
Arthroscopy and joint biopsy was done and pigmented villonodular synovitis was reported in pathology. The surgeon had observed a gray-black tissue which had replaced in synovium and cartilage. He undertook open surgery and black synovium and cartilage and bone were removed. Microscopically, sections of fibroconnective and synovium tissue revealed gray foreign bodies which were ingested by gient cells with lymphocytic infiltration seen in background field. Prussian blue and Zeihl-Nelsen stainings were negative. We suggest Ochronisis (Alkaptonuria) in pathology report. Benedict test as screening and change in urine colour after 1 hour were positive. Finally gas liquid chromatography confirmed Alkaptonuria as the definitive diagnosis.

P29

Curious Lump in the Thigh of a Young Male

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We present an unusual case which to the best of our knowledge (based on literature search) has never been reported before. A skin biopsy was received in February 2008 from a 20 year old male. The clinical description was "hard scar in the right lateral thigh in the deep dermis and subcutaneous plane ?fibroma. Has grown with the patient. Clinically the area is firm to hard." Histological examination revealed a dermal proliferation of bland spindle cells not unlike a hyperplastic scar except for the presence of epithelioid cells, some of which showed mild cytological atypia and demonstrated slightly more intensely eosinophilic cytoplasm than the surrounding spindle cells. These large cells were scattered within the deep dermis and subcutis either individually or in very small nests. No mitotic activity was noted. Immunohistochemical staining showed the large cells to be positive with cytokeratin 7 but not with CK20, AE1/3 or MNF116. EMA was negative. The spindle cell were focally positive for S100. Also, in areas, nerve fibres were highlighted by both S-100 protein and PGP9.5, the latter stain highlighting some of the spindle cells in the infiltrate. Neurofilament faintly stained the fibres mentioned above as did Glial Fibrillay Acidic Protein. The features favoured a neural lesion but it was difficult to explain the cytokeratin positivity except if it was was put down as aberrant expression. Despite a literature survey and extensive local consultation a definite diagnosis could not be reached. Given the unusual morphology and immunophenotype (with cytokeratin positivity) the case was referred to Dr E Calonje and Prof CDM Fletcher for specialist soft tissue pathology opinion. They both reported having seen nothing similar before but concurred that it was most likely a benign neural neoplasm/hamartoma or localised anomaly that should be completely excised. The patient is well two years post excision.

P30

Trends in Primary Cutaneous Melanoma: A 10 Year Single Institution Review

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Purpose: To retrospectively analyse all primary cutaneous melanomas submitted to the Histopathology Department over a ten year period (2000-2009). Our aim was to determine disease incidence and demographics and identify changing trends in presentation including a possible seasonal variation in tumour distribution. Methods: A review was conducted within our institution (University affiliated teaching hospital, catchment area 290,000) to identify all primary cutaneous melanomas resected between 2000 and 2009. The reports were audited using agreed dataset criteria including: age, sex, month of resection, site, subtype (nodular, superficial spreading, lentigo and other) and Breslow thickness. The data was analysed in spreadsheet format using Excel statistics. Results: We identified 263 cases in the defined study period. Females outnumbered males in 1.66: 1 ratio (n= 164: 99) with a predominance of patients > 50 (n= 167, mean age at presentation 58.5 years). The number of cases per annum increased between 2000 (n=19) and 2009 (n=40) with an increase in male cases from 21% (n=4) to 57.5% (n= 23). A seasonal peak in presentation was seen in summer with an overall summer:winter ratio of 2.39. Additionally, melanomas presenting over the summer had a greater Breslow thickness than those over the winter months (summer: winter ratio of 2.86 for those > 4mm). Conclusions: Our study has identified three interesting trends. The first is confirmation of the seasonal variation in presentation and thickness of melanomas in concordance with other international studies. The second is documentation of the increasing incidence of the disease over a decade of study and the third is the increasing number of male patients presenting with this tumour. This is the first study within Ireland to subclassify the distribution of primary cutaneous melanoma with summer: winter ratios based on patient and tumour characteristics.

P31

An Audit of Subtyping Basal Cell Carcinoma in Diagnostic Biopsies

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Basal cell carcinoma (BCC) is the most common type of invasive skin malignancy. Pre-operative biopsy is recommended when there is a clinical doubt or a specialized treatment is required. In addition to the risk of recurrence, identification of a high risk histological subtype is important to reduce the local destructive potential of the tumour. The aim of the audit was to identify the percentage of BCC reports which recorded the histological subtype in diagnostic biopsies in our department. The accuracy of subtyping was also assessed by comparing with subsequent excision biopsies. The search for all BCC cases reported for one year was achieved electronically using the SNOMED coding system on telepath. This search revealed 847 BCC reports. 94/847 BCC reports of diagnostic biopsies with subsequent excision biopsies were identified by manual search. 11 cases were excluded as there was no residual BCC or the subtype was not recorded in the subsequent excision biopsies. The final number of cases included was 83. The subtype in diagnostic biopsies was reported in 61/83 (73.5%). Diagnostic biopsies correctly identified the BCC subtype in 47/61 (77%) cases. A total of 22 cases (26.5%) were not assigned a subtype on diagnostic biopsy. Of these 50% (11/22) were found to be a high risk subtype on subsequent excision biopsy. These cases included 7 punch biopsies, 2 curettings, 1 shave biopsy and 1 case where the biopsy type was not specified. In conclusion, the accuracy of histological subtyping in diagnostic biopsies was high (77%) and similar to published data, however, it was recognised that failure to report a high risk subtype in diagnostic biopsies may have a direct impact on the prognosis.

P32

Human Dirofilariosis in a 53 Years Old Female Patient: An Uncommon Entity in the Alpine Region

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Introduction: Dirofilaria repens and Dirofilaria immitis are well known parasites, which are endemic in the climate conditions of Europe. Their natural hosts are carnivores (e.g. cats, dogs). Infection is transmitted by mosquitoes. Humans get accidentally infected, showing subcutaneous dirofilariosis in most cases. Dirofilaria repens and immitis are not endemic in the Alps. Patient Presentation: A 53 years old female patient, who lives in Southern Tyrol, was biopsied due to a re-occurring swelling of the right parotic region in an outward hospital. Radiological examinations were unspecific. Outward histological examination of the specimen showed a non-specific inflammatory reaction. In fact the patient was treated in a conservative way. Due to persisting symptoms the patient was presented at the University Department of Dermatology, and due to being suspicious for sialadenitis transferred to the Department of Dental Surgery, where she underwent a surgical biopsy. Parasite infection was diagnosed by histology. The diagnosis Dirofilaria repens was confirmed by PCR analysis. No recent travel history was positive for Dirofilaria spp. Conclusion: Human Dirofilariosis is a parasite infection rarely seen in the Alpine region. Between 1981 and 2008 only 13 cases of subcutaneous Dirofilarosis have been reported in Austria. Climate changes are discussed as possible source for increasing infections in central Europe. Although most unlikely in our region, the possibility of a parasite infection has to be excluded in unclear subcutaneous swellings. Histology and PCR-Analysis are mandatory for diagnosis.

P33

MELAN-A Expression in Atypical Fibroxanthoma and Pleomorphic Dermal Sarcoma

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Atypical fibroxanthoma (AFX) is a solitary dermal-based tumour that primarily occurs on sun-damaged skin of the head and neck area of the elderly. If confined to the dermis behaviour is benign following complete excision. Invasion of deeper structures is associated with risk for local recurrence and those tumours are best regarded as pleomorphic dermal sarcomas. The diagnosis is one of exclusion using a broad range of immunohistochemical markers. We report three patients with AFX/pleomorphic dermal sarcoma showing aberrant expression of MELAN-A. All three tumours were dermal based and composed of plump spindle cells with admixed large bizarre giant cells in varying proportions. All tumours involved the subcutaneous fat and in all cases, MELAN-A was focally expressed in large giant cells. Tumour cells stained positively for CD68, CD10 and focally for SMA. They were negative for desmin, multiple cytokeratins, CD31, CD34, S100 and HMB-45. S-100 protein expression was noted only in scattered dendritic cells. Aberrant expression of MELAN-A in AFX/pleomorphic dermal sarcoma represents an important diagnostic pitfall, particularly when tumours invade beyond the dermis similar to desmoplastic or spindle cell melanoma. Expression of MELAN-A limited to the giant cells and the absence of S-100 are helpful clues to the diagnosis, as is the fact that expression of second line melanoma markers in desmoplastic melanoma is rare.

P34

Audit of Incomplete Excision Rates of Basal Cell Carcinomas

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Purpose- Incomplete excision of basal cell carcinomas (BCCs) can have significant impact on morbidity. As part of reflective practice and improvement in service, an audit into the rate of incompletely excised BCCs by various clinical groups was caried out. This would help identify the need for imrovement in current practice and allow maintenace of clinial standards.The data would also identify factors associated with an increased risk of incomplete excision and correlate with the current literature. Criteria and standards : Various regional audits and literature from the British Association of Dermatologists and have shown acceptable incomplete excision rates to range from as low as 4.7% to as high as 13%. From such existing data, a local agreement of the minimum standard of incomplete excision was established and a minimum requirement of 13% was set as the standard. Method: Data was derived retrospectively including up to seven regional hospitals. 1246 reports on BCC skin excisions were analysed according to their gender,clinical groups, tumour site, tumour size, histological subtype and completeness of excision. Results and Conclusions: The overall excision rate of incomplete BCCs was found to be low at 4.3% and no change in management was adviced. Factors asociated with an increased risk of excision included the following; tumours arising from the periorbital region, scalp and ear; Tumour size greater than 1 cm; histological subtype of micronodular, morphoeic and mixed subtypes; excisions performed by general practice.

P35

Co-existent Granulomatous Vasculitis and Leukaemia Cutis in Resolving Herpes Zoster

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An 80-year-old man with a 5 year history of small lymphocytic lymphoma (SLL) being managed conservatively under a "watch and wait" protocol, subsequently presented with a six month history of indurated tender purple papules. These coalesced to form plaques with some central scarring, and had a dermatomal distribution on the left arm, immediately following herpes zoster infection at this site. Skin biopsy showed marked interstitial granulomas and prominent granulomatous vasculitis, supporting the clinical impression of a post-herpes zoster granulomatous reaction. In addition, there was a dense monoclonal small B-cell lymphocytic infiltrate indicating Koebnerisation by SLL - a finding which previously has not been reported with concurrent post herpetic granulomatous vasculitis. Although benign pseudolymphomas occur in post-herpetic settings, this case shows that even in association with benign vasculitic features true lymphomas occur. Furthermore it highlights the value of the appropriate use of detailed immunohistochemical analysis, and the importance of clinicopathological correlation.

P36

Detection and Quantification of MicroRNAs in Laser Microdissected Formalin-Fixed Paraffin Embedded (FFPE) Breast Cancer Tissues

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MicroRNAs (miRNAs) are a class of endogenous non-coding RNAs that target protein coding mRNAs for cleavage or translational repression. Both profiling and functional studies demonstrate deranged miRNA expression in many human cancers including breast tumours. Research in this field is increasing and the potential of miRNAs for being used in clinical settings emphasises the need for sensitive detection techniques. In this study, techniques for the analysis of miRNA expression in microdissected FFPE breast cancer tissues were developed and optimised. Full face sections from three invasive breast tumour samples and different microdissected areas (1,000-10million ìm2) and section thickness (10-20ìm) were analysed. Total RNA was extracted using commercially available RNA extraction kits (miRNeasy FFPE Kit, Qiagen; RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE, Ambion; PureLink FFPE RNA Isolation Kit, Invitrogen). Three miRNAs (miR21: highly expressed, miR-29c: intermediately expressed, and miR-127: low expression in breast cancer) extracted from both gross and microdissected invasive breast cancer tissues were quantified using real-time PCR. The PureLink kit produced largest quantities of total RNA from FFPE breast tumours. All three miRNA (21, 29c and 127) were successfully detected by real-time PCR and levels of sensitivity were comparable between extraction methods. Our data showed that relative miRNA levels gradually decreased with diminishing amounts of microdissected tissue used but reliable miRNA quantification was obtained using at least 5 million ìm2 from 20ìm thick FFPE breast tissue sections. In contrast to previously published results, quantity of miRNA detected in breast tissue samples depends on the amount of tissue used, and cannot be performed reliably from one or a few cells.

P37

Suppression of Tissue Autofluorescence with Copper + Hydrogen Peroxide

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The simultaneous immunohistochemical staining of multiple tissue antigens has largely been the preserve of immunofluorescence. In addition, it is probably the only technique that facilitates multiple staining for confocal microscopy. Tissue autofluorescence remains a serious problem, however, and is particularly troublesome where low levels of antigen require illustration. A number of techniques have been developed to suppress tissue autofluorescence such as treatment with copper sulphate, or masking of specific fluorescent substances, such as lipofuscin, with dyes. Here we describe the use of copper sulfate + hydrogen peroxide (a technique that was originally developed to suppress tissue argyrophilia) for suppressing tissue autofluorescence. To evaluate the technique, 4µm sections of paraffin wax-embedded 1% glutaraldehyde-perfusion fixed rat kidney were treated with 10mM copper sulfate or 10mM copper sulfate followed by 3% hydrogen peroxide. Compared to untreated tissue, copper treatment produced a noticeable reduction in autofluorescence, but when combined with subsequent treatment with hydrogen peroxide, a further and dramatic improvement was observed. To evaluate the utility of this technique in immunofluorescence staining, further sections of rat kidney were pre-treated with copper + hydrogen peroxide and immunofluorescently stained for aquaporin-1. Staining was compared with untreated sections and with post-staining treatment with copper. In untreated sections, positivity was barely discernable against the intense background autofluorescence. In contrast, immunopositivity could be clearly seen in tissue sections that had been pretreated with copper + hydrogen peroxide. Post-staining treatment with copper suppressed autofluorescence, but also reduced immunofluorescence.

P38

Raman Microspectroscopic Mapping of Thin Histopathological Tissue Sections

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Disease diagnosis of a biopsy sample is performed routinely by the examination of stained tissue section under a light microscope. Raman spectroscopy shows considerable promise as an optical technique for disease diagnosis, due to its ability to provide valuable biochemical information from tissue samples at the molecular level. As most pathologists and other clinicians are not spectroscopically trained, Raman spectroscopy will only be useful to them if there is a way to present the thousands of Raman signals, obtained from different parts of a tissue section, in a format that is easily interpretable i.e. a Raman map. Paraffin-embedded clinical tissue samples were obtained from the archival files of the Pathology Department, Ninewells Hospital, Dundee with ethics approval from the Tayside Tissue Bank. 4-6μm thick unstained sections were cut and placed onto quartz slides and de-paraffinsed. Raman mapping was performed by raster scanning the tissue using a home-built Raman system. Multivariate analysis was performed by hierarchical cluster analysis (HCA) of the Raman spectra associated with the different tissue types and Raman maps were generated using the resultant cluster. Using normal cervical tissue, we successfully mapped squamous epithelium and the epithelial-stromal interface, muscular artery and endocervical glands. Analysis of a tissue section containing a cervical intraepithelial neoplasia (CIN) grade 2 lesion adjacent to normal squamous epithelium demonstrated that the CIN lesion clustered predominantly with the basal epithelial cells of normal epithelium and allowed visual discrimination of these areas using the Raman map. These findings suggest that Raman mapping has the potential to provide images that are useful for disease diagnosis. In particular, the discrimination between normal cervical squamous epithelium and CIN is of relevance to cervical screening pathology.

P39

Abstract Withdrawn

P40

Virtual Microscopy Meets the Microsoft Surface: a Multi-Touch Experience for Digital Pathology

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Virtual microscopy provides enormous opportunities for supporting educational, research and diagnostic applications in pathology. Traditionally, whole slides scans are viewed using a standard computer interface, where slides are navigated on a computer monitor using a mouse or keyboard. The Microscoft Surface provides an alternative multi-touch software interface where software control is through natural figure movements and hand gestures across the table surface. The aim of this study was to explore the Microsoft Surface and development of multitouch control for virtual microscopy. We developed a software interface which allows high resolution virtual slides to be viewed on the Microsoft Surface. This was built using the Surface SDK on the .NET framework using Surface libraries and integrated with PathXL (i-Path Diagnostics) for image serving. The virtual slide can be navigated in x and y, and focussed using finger movements across the touch sensitive surface. By swiping a finger from left to right the virtual slide will move to the left. By positioning two fingers close together and dragging outwards, the image magnification will increase. Annotations can be drawn by hand allowing convenient marking of key morphological observations. These natural movements make the use of virtual slides more intuitive. Controls also allow multiuser interaction locally, where several users can sit around the table and discuss a slide, and also for remote consultation. While education in pathology would be the biggest beneficiary of this technology we are already implementing a tissue microarray interface which allows automatic core identification and where image analytic algorithms can be integrated seamlessly for the manual or automated analysis of virtual slides. This novel multitouch approach to virtual microscopy represents an exciting and more intuitive approach to virtual microscopy across a range of applications.

P41

PCR Based Tissue Identification- the UCLH Experience

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PURPOSE OF THE STUDY Accurately identification of tissue from an individual is crucial in the laboratory if mislabelling of slides or carryover of samples is suspected. Equivocal samples, including sub-divided fragments can be identified by PCR amplification of simple tandem repeat (STR) sequences. Here we report our experience of this technique from 2003-2010. METHODS PCR was performed using primer sets designed to target small fragments covering STR regions, to permit analysis of degraded DNA samples and products analysed on polyacrylamide gels. All submitted cases for UCLH patients were reviewed and their contribution to patient management assessed. SUMMARY OF RESULTS Eighteen cases, including one from cytology were included. Six cases (33%) were of clinically significant 'carry over'. Five of these were confirmed as having extraneous tissue present on the slide and the provenance of one of these cases was identified. In 7 cases (39%) a mix-up, either of forms and tissue, of tissue alone or of mislabelled slides was suspected. In all cases the analysis identified tissue to an individual and resolved the mix-up. In 2 cases prostatic carcinoma was diagnosed in young individuals. At the patient's request, peripheral blood and the corresponding tissue samples were compared in order to confirm identity. In both cases the tissue was correctly labelled. In 3 cases two consecutive biopsies showed a significant histological discrepancy, but PCR confirmed that no mistakes had been made. CONCLUSIONS The PCR STR analysis was reliable, robust and easy to perform in cases where the origin of tissue samples was in doubt. The results enabled improved patient management with consequent cost saving and quality control benefits.

P42

Epithelial Mesenchymal Transition: Does it happen in Gastric and Oesophageal Cancer?

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Epithelial Mesenchymal Transition (EMT) is a proposed mechanism for cancer metastasis and is characterised by the acquisition of mesenchymal markers, vimentin (VIM) and n-cadherin (CDH2), and the loss of epithelial markers, e-cadherin (CDH1) and cytokeratin (CK). In the upper gastrointestinal tract information on the occurrence of EMT is very limited. Our research aimed to investigate the frequency of EMT in gastric adenocarcinomas and oesophageal squamous and adenocarcinomas in relation to clinicopathological data.

We used immunohistochemistry to evaluate CK and VIM expression in 31 full sections as well as in tissue microarrays of 368 gastric adenocarcinomas (GC), 108 oesophageal carcinomas (OeC) and 26 GC cell lines. In addition, 31 GC full sections, 52 squamous OeC and 26 GC cell lines were stained for CDH1 and CDH2.

23% GC cell lines expressed VIM compared to 2% primary GC. A complete EMT with VIM/CDH2 positivity and reduced CK/CDH1 was found in 12% of GC cell lines but not seen in primary GC. VIM expression was significantly higher in squamous OeC than in adenoOeC (65% vs. 14% of cases; $p<0.001$) and complete EMT occurred in 8% of squamous OeC. No relationship with clinicopathological data or survival was observed.

This is the first study to demonstrate (i) a higher rate of EMT in GC cell lines compared to primary GC which may be related to the artificial *in vitro* environment of cell culture and (ii) a higher rate of EMT in squamous cancer compared to adenocarcinoma in the upper GI tract. The latter may suggest that EMT is regulated differently depending on morphological subtype. These findings need to be confirmed in a second independent large series and extended to small and large bowel cancers.

P43

P53 Dependent Repression of PLK1

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We investigated the repression of PLK1 in a number of cell lines. PLK1 is an important regulator of the cell cycle as reflected by its expression. PLK1 is expressed as low levels at G1, increasing into S phase and becoming most elevated in G2/M where it acts as a checkpoint to allow G2/M transition. In addition, P53 is required for maintenance of the G2/M checkpoint and without P53 cells are pushed into mitosis. Previous research has shown that as P53 levels augment, PLK1 decreases in expression, however the mechanism for this remains unclear. We have examined the effects of DNA damage in cells and confirm that PLK1 expression is repressed following P53 up regulation. In addition successful knockdown of P21 by siRNA has shows that PLK1 depletion is independent of P21. Furthermore, we find that P53 is recruited to the PLK1 promoter under conditions of etoposide induced stress and when linked to a luciferase reporter gene, PLK1 promoter activity is repressed. These findings suggest that at the G2/M checkpoint, PLK1 is suppressed in a P53 dependent manner. Over expression of PLK1 is often observed in tumour cells and consequently may offer therapeutic interest.

P44

Variability in X Chromosome Inactivation Patterns in Multiple Tissues of Elderly Females.

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Every female somatic cell has 2 X chromosomes, one paternally (Xp) and one maternally derived (Xm). In order to reach an approximately equal dosage effect of genes from each X, one is inactivated in an apparently random manner. Most females show a slight detectable bias towards one X, a small minority showing a marked preference. Significant bias is termed skewing. Acquired age related skewing in peripheral blood has been confirmed by several studies.

Secondary selection may occur when a mutation on the either the Xp or Xm confers a survival advantage to a stem cell or its projeny; conversely if the mutation is detrimental, cells bearing that X will be selected against. As well as determining phenotype in X-linked disorders, skewing influences several renal diseases and may be implicated in autoimmune diseases such as scleroderma and systemic lupus. Increased skewing rates have been linked to recurrent pregnancy loss, pre-eclampsia and autism. The role of acquired or chance skewing as a risk factor for malignancy is currently under investigation.

To date most of the studies of X inactivation have been on peripheral blood with a few investigating other relatively accessible tissues such as hair bulb epithelium. We chose to investigate ratios in females >60 years of age in which age related skewing was more likely to occur.

Our results show a general concordance between most tissues of the same individual with skewing being evident in the spleen of 2/11 and in the liver of 3/11 females. This study suggests that age related skewing may occur in the liver as well as the haematopoietic system This is the first study looking specifically at skewing ratios in multiple inaccessible tissues of elderly females and the first case reported of skewing in both the liver and spleen of an elderly female. These organs skewed in the same direction supporting the growing evidence that skewing is a selective phenomenon

P45

Cytotoxicity of Novel Gold (I) Stable N-Heterocyclic Carbenes Against Human Tumour Cell Lines.

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Cisplatin has proved useful as a cytotoxic drug for cancer chemotherapy but also exhibits nephrotoxicity and neurotoxicity. Thus the potential of other metal compounds needs investigating. A series of novel gold stable N-heterocyclic carbenes were synthesised and tested for cytotoxicity using both established human tumour cell lines and normal and tumour lines derived from the same patient. Human cell lines derived from prostate (LNCaP, P21TZ and P21PZ), breast (MDA MB231, B42 and B42 clone 16) and bladder (SV-HUC-1 and MGH) were compared. The cytotoxicity was assessed in 96 well plates using a cell proliferation assay. Structural changes in the gold-NHC compounds by altering the terminal groups resulted in different cytotoxic responses of the compounds. There were no marked differences in cytotoxicity in the active compounds when normal and tumour lines were compared. The normal urothelial cell line SV-HUC-1 was compared to the bladder tumour line MGH. Normal mammary epithelial cells (B42) and normal prostate epithelial cells (P21TZ) were compared to respective tumour lines from the same patient (B42 clone 16 and P21PZ). The bladder cell lines were more sensitive than the established prostate (LNCaP) and mammary cell lines (MDA MB231). These novel gold compounds exhibit cytotoxicity in vitro with differences based on their structure. The compounds that exhibited cytotoxicity to all the different cell types had a hydroxyl or a chlorine group bound to gold.

P46

Properties of Exosomes Derived from Normal and Malignant Prostate, Breast and Bladder Cell Lines.

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Exosomes are small vesicles 50-100 nm in size that are secreted from both normal and tumour cells. They have a wide ranging activity being able to modify the immune response to tumours and having the potential to act as tumour markers. Exosomes were isolated in the supernatants from normal and tumour cell lines. They were concentrated by centrifugation, firstly by collecting the supernatant after a 10,000g spin then pelleting the exosome rich fraction at 100,000g. The exosomes were further characterised by FACS analysis following exposure of the cells to carboxyfluorescein succinimidyl ester (CFSE) and to immunoblotting for exosome markers. The exosomes express Tsg101 and ALIX detected by immunoblotting. These are well characterised exosome markers. FACS analysis following calibration with beads revealed that the particles exhibited a size consistent with that reported for exosomes. The particles in this size range were labelled with CFSE indicating that they had been synthesised by the cells. Fetal calf serum was depleted of exosomes by differential centrifugation. The exosome fraction expressed the markers Tsg101 and ALIX. The growth rates of mammary epithelial cells and the MDA MB231 breast tumour cell line were decreased in exosome depleted medium. The ability of prostate tumour cells to attach to prostate fibroblasts was also compared in exosome depleted medium and with exogenous exosomes added. The ability to attach was modified by the exosomes. Exosomes derived from tumour cell lines modify the growth and attachment of tumour cells.

P47

Non-Technical Skills in Histopathology: Definition, Relevance and Potential Application

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The NHS is a high risk organisation where there is a 10% possibility of adverse events for hospital patients. Most safety incidents in health care and other high risk industries like aviation are associated with "human factors", failures in cognitive and social skills, classified as non-technical skills, comprising situation awareness, decision making, communication, team-working, leadership, managing stress and coping with fatigue. We explore how non-technical skills are relevant to histopathological practice where these risks apply. Histopathologists make hundreds of decisions that affect patients. Getting these correct requires extensive training and professional development, audit and EQA. While these processes improve performance, they may not be sufficient to ensure a high level of safety. Delivery of high quality care requires more than diagnostic acumen. Communication between medical, scientific and administrative staff in laboratories is crucial, as is that with multidisciplinary care teams. Clarity within departments about leadership responsibilities and accountability is necessary. These factors are acutely relevant in the stressed environments in which many histopathologists work. The aviation and nuclear power industries have recognised the need to understand the factors influencing human performance in safety critical occupations. They have identified non-technical skills that are protective for safety and train them to reduce the likelihood of human error. Anaesthesia and surgery have recently developed non-technical taxonomies relevant to their practice leading to training to assist skill development. This paper explores how developing non-technical skills in histopathology could enhance our ability to provide a safe accurate service, help us to construct training that is more safety-sensitive and improve the quality of our practice.

P48

Cognitive Errors in Histopathology

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Glass slides are the main source of diagnostic information for histopathologists along with the given history and gross appearance. The processes of examining and interpreting sections present challenges for histopathologists that we will explore from a cognitive psychology perspective. Histopathology is vulnerable to overcalls and missed diagnoses. Some divergences of diagnostic opinion emerge from cognitive rather than medical errors, more specifically from our natural "Cognitive Dispositions to Respond" (CDRs; Croskerry, 2003), i.e., mental shortcuts that often serve us well but occasionally fail.
To reduce cognitive errors, we need to understand why we have particular CDRs in particular clinical situations and how these behaviours are relevant to diagnostic practice. We will explore and demonstrate the relevance of the following CDRs as they pertain to histopathology: a) availability, which leads to judging a diagnosis as more likely if one had recent experience with it, b) confirmation bias, which leads to searching only for confirming evidence to support one's diagnosis, c) premature closure, which leads to accepting a diagnosis before it has been fully confirmed and, d) search satisfying, which leads to a halt in searching the sections once something is found. We consider these potential cognitive errors and how they could arise in a histopathologist's everyday practice. The domain seems amenable to experimentation that is currently lacking but could reveal conditions enhancing error. We propose that cognitive psychology can enrich our understanding of the diagnostic process further and has potential value in enhancing diagnostic safety. Histopathologists as medical practitioners and educators would benefit from realising the contribution of cognitive errors to diagnostic errors and how the impact of these cognitive errors could be reduced by raising awareness as a first step.

P49

Emergency Requests for Histopathology–Standard Setting and Audit

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We noted high numbers of apparently inappropriate "urgent" requests for histopathology reports using time and diverting resources. Many lacked clinical details justifying the "urgency" and contact details. We sought to establish the nature of these requests and thus try to change behaviours in requestors. We identified 102 consecutive urgent requests (Series 1, S1; September - October 2008) and collected details of these electronically. Results were fed back to requestors. Over the same time interval in 2009, we carried out an audit following the intervention using the same methods (Series 2, S2), collecting 76 cases. This study presents our findings.
Most specimens in both series were gastrointestinal biopsies although many other specialties were represented. Clinical contact details were absent in 59% of S1 and 47% of S2 cases and in many clinical details were scanty. Specimens spent between <24hours to 5days to reach the department. Cases were reported by consultants alone in 54% (S1) and 53% (S2). Telephoned reports took on average 1day 18hours (S1) and 2days 8hours (S2). Trainee involvement did not affect this. Clinical diagnosis varied between S1 and S2: malignant 38%, 86%; benign 19%, 5%, inflamed 43%, 4%, none given 0%, 5%. Pathological diagnosis also varied: malignant 25%, 57%; benign 32%, 37%; inflamed 37%, 11%; not diagnostic 1%, 0%.
Overall, there was a good correlation between the clinical and pathological diagnoses ($p<0.0001$) although only 64% of clinically malignant cases were classed as such pathologically. Discussion with clinical colleagues resulted in fewer urgent requests over the same time period one year later and a higher proportion of possibly malignant cases in this group. Concluding, we find that feedback can influence requesting behaviours. We concede that there is still room to improve the provision of contact details and clinical justification of urgent requests.

P50

National Framework for Induction & Introduction to Histopathology (England/Wales) – Strand A: An Audit of the Induction Period, ST1 Trainees' Perspective.

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The induction is fundamental to ensuring effective transition for specialist trainees into new roles and departments. PMETB and the MMC both set out standards for trainee induction. The aim of this audit is to compare the current ST1 induction in histopathology with these standards, from the trainees' perspectives. This audit is being carried out in parallel to one looking at induction from the trainers' perspectives.
A questionnaire was created using 'Survey Monkey', covering the PMETB and MMC standards for induction and distributed to all current ST1 histopathology trainees in England and Wales.
The response rate was 40.6%. 96% of trainees had a departmental induction. The majority of the aspects of induction included in the standards were received by >80% of trainees. The two standards for which <80% of trainees received induction were provision of information about the service the trainee is working within and provision of information about when RCPath events were. For the majority of the standards audited, >85% of trainees felt the information provided relating to that standard was adequate or excellent. The areas where ≤15% trainees felt induction was poor were information about support systems available; information about the service they were working within; information about the on-line portfolio and information about when RCPath events were.
This audit indicates parts of the induction period that could be improved to bring it in line with national standards. Introduction of a national framework for induction in histopathology would provide consistent induction across the SHO training schools, incorporating the PMETB and MMC standards and focussing on the specific needs of new ST1s in histopathology.

P51

National Framework for Induction & Introduction to Histopathology (England/Wales)–Strand B: An Audit of the Induction Period from the Trainers Perspective

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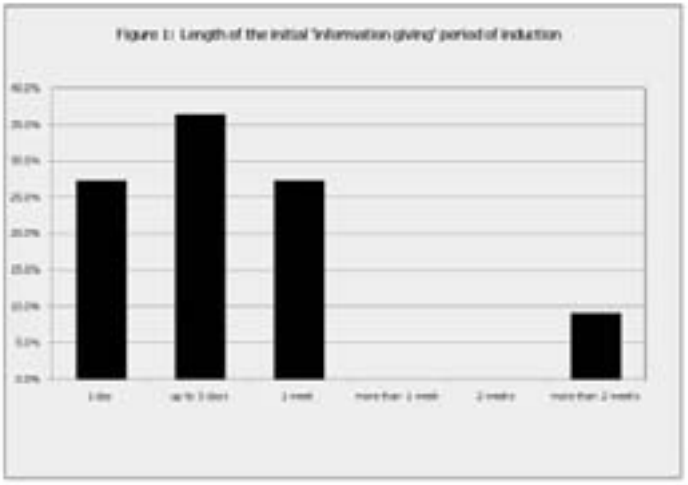
Histopathology school leads are required to provide induction for all ST1 trainees.
The purpose of this audit is to compare current practice for induction, from the trainers perspective, against the minimum requirements set out by PMETBs Generic Standards for Training[1] and the MMC Gold Guide[2], to ascertain if criteria are being met. A parallel audit (strand A) has looked at induction from the trainees point of view.
A questionnaire was created within Survey Monkey (www.surveymonkey.com) and sent to school leads in England and Wales.
The response rate was 57.9%. 100% felt a robust induction programme was in place. Induction takes place on the first day in 27.3%, within the first week in 63.6% and within the first month in 9.1% (see figure 1 for the length of induction). Out of the ten topics which are PMETB/MMC mandatory requirements for induction six of them were being covered by 100%, two by 90.9%, one by 54.5% and one by 45.5% of schools. After the initial induction 54.5% put trainees straight into diagnostic teams. 81.8% felt that if induction were lengthened it would negatively impact on curriculum delivery.
Although all schools felt that they had a robust induction programme in place, this audit highlights that some PMETB/MMC requirements are not being covered and that induction practises and trainees experiences after the initial induction vary between schools. We recommended a national framework for induction be created, incorporating PMETB/MMC standards, to guide school leads so as to standardize the induction period and ensure all trainees receive a good quality induction. It is hoped this will also cut down time trainers need to spend preparing induction.
References
1 PMETBs Generic Standards for Training
2 A Reference Guide for Postgraduate Specialty Training in the UK – Gold Guide (2009)



P52

Perceptions of the Teaching of Pathology Amongst Medical Students: A Comparison Between a Problem-Based and a Traditional Lecture-Based Learning?

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Purpose of Study: The reform of undergraduate medical teaching has led to an integrated problem-based learning (PBL) style replacing the traditional lecture-based (TLB) learning. The Royal College of Pathologists state "the low profile of pathology teaching in the undergraduate medical curriculum has become increasingly damaging to the recruitment of quality UK medical students." This study evaluated the perceptions of Pathology teaching amongst medical students from both PBL and TLB medical schools. Methods: An on-line survey was sent to medical students from 24 medical schools. They were asked about the quality of Pathology teaching (including what facilities were provided) at their medical schools and their understanding of Pathology. Summary of Results: 1126 students from 17 universities responded, with even representation over years 1-5, the data was analysed using the Mann-Whitney-U test. Approximately 75% of students from PBL courses compared to 38% from TLB courses said Pathology teaching was neglected ($p<0.001$) on their curriculum and only 20% (PBL) versus 52% (TLB) felt they had a good understanding of Pathology ($p<0.001$). Approximately 10% (PBL) and 17% (TLB) said they were specifically examined on Pathology during their end-of-year/final exams. The TLB courses provided far superior teaching facilities compared to PBL, e.g. 63% providing microscopes compared to 11% of PBL courses. Furthermore, 74% (TLB) practiced cadaver dissection compared to only 24% of PBL courses, 34% (TLB) of which had some formal pathology during these sessions compared to only 6% in PBLs. Conclusions: Pathology underpins a majority of clinical diagnosis and decisions that are made. It's understanding (through effective teaching) is paramount to good medical practice. This study demonstrates that Pathology is poorly assessed in medical exams and more worryingly that PBL is poor in disseminating pathological principles.

P53

Students' Deep, Strategic and Surface Approaches to Learning and Studying Over a Medical Degree Programme

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Students combine three approaches to learning and studying: deep, where learners aim for long term retention of subject matter thorough understanding; surface, which implies learning by rote with little understanding; and strategic, which concentrates on achieving high grades. Learning approach may change with environment. We used the Approaches to Study Skills Inventory for Students (ASSIST) questionnaire to measure the scores of medical students. We previously showed little change between early first year and late second year, with a fall in surface but little significant change in deep or strategic. Using electronic data collection, we have now extended the study to include students in later years of the medical degree programme.

Reliability (Cronbach alpha) was >.7 for scales and >.5 for subscales. At the start of year 1 students in both cohorts gave high scores for deep and strategic, but relatively low scores for surface (Tables). Spearman correlation of matched pairs between first and later years showed no significant increase in scores for deep and strategic and a slight but not significant fall in surface. This slight tendency for surface scores to decrease is in keeping with results from studies in other subject areas at university.

Various factors could explain the findings. Students enter with established approaches, scoring highly on deep and strategic approaches and it may be difficult to increase the scores further. Assessments were deliberately designed to test for competence and may not sufficiently promote a deep approach. Some students may succeed in assessments by using a surface approach and see no need to change. Finally, the questionnaire may not detect changes in approaches in learning and studying in medicine, either because some questions are inappropriate or do not reflect students' real study practice.

P54

Attitudes of First Year Medical Students from Different Religious Backgrounds to the Autopsy

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Introduction: The role religion plays in shaping attitudes towards the autopsy is an important one and can impact the way in which doctors take consent for autopsies. Conversely doctors can also face resistance from bereaved relatives to autopsy based on religious grounds. Aims: To investigate the attitudes of first year Muslim, Christian and Atheist medical students to establish whether religion affects their views on the autopsy and their thoughts about taking consent from bereaved relatives after qualification. Methods: First year medical students responded to an advert on a medical student intranet site. Three semi-structured focus group discussions of the respective groups were audio taped. Their religious views, personal experiences of autopsy, the role of the coroner and taking consent for autopsy were discussed. Recordings were transcribed verbatim and subjected to a qualitative analysis by defining codes and sub-codes. Subsequent collation of the data allowed direct comparisons to be made between the three groups. Results: Most participants felt uncomfortable with the physical procedure of the autopsy, especially on a member of their own family. Findings from the Christian and Atheist groups were very similar with no objections to autopsy based on their religious or personal beliefs. The Muslim group believed that the deceased should be buried immediately and were concerned an autopsy would delay this religious obligation. Discussion: This study demonstrates that religion affects the personal beliefs of students and influences their behaviour in their personal lives. They are aware that their beliefs could infringe on their professional responsibilities as doctors, and their interactions with patients of other beliefs. They are keen to learn about other religions so that they can manage those interactions.

P55

The Delivery of Pathology Teaching in the Medical Curriculum: How Often are Medical Students Taught and Examined in 'Pathology'

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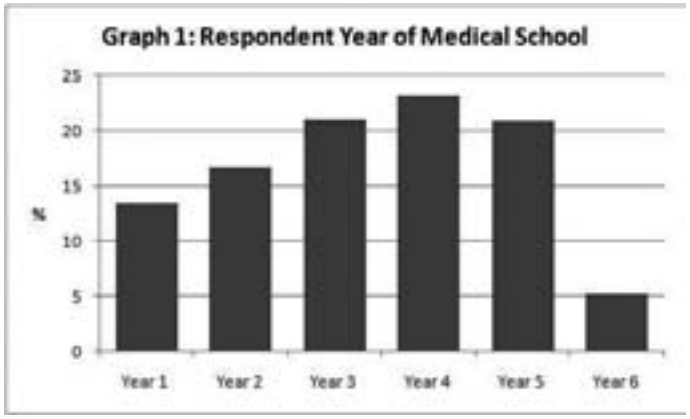
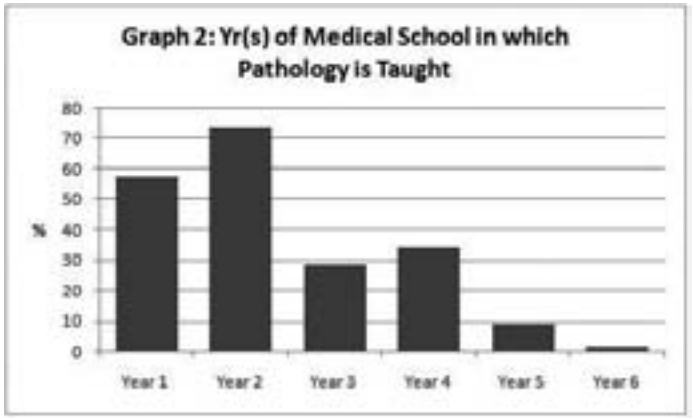
Introduction: The Royal College of Pathologists (RCPath) response to the 2007 NHS Employers paper 'The Future of the Medical Workforce' stated "*lack of exposure to modern scientific principles during undergraduate... medical training results in a total workforce that is significantly deficient in a modern comprehension of pathological processes*" and that "*the low profile of pathology teaching within the undergraduate medical curriculum has become increasingly damaging to the recruitment of quality UK medical students.*" Leading on from these statements an audit group from the RCPath sought to determine at what stage and how often medical students were taught pathology as a surrogate marker for the extent and profile of pathology teaching and whether pathology is formally examined in end of year and final examinations as a surrogate for sufficient comprehension of pathology.

Methods: An on-line survey was sent to Medical Students in all years of study from 24 medical schools in England and Wales. Students were asked to provide information regarding the frequency and timing of pathology teaching and examinations at their medical school.

Results: Responses were received from 1126 medical students from 17 medical schools in England and Wales, distributed between years 1-6 (Graph 1). Students reported pathology to be predominantly taught during years 1 and 2 (Graph 2) and only 4% reported pathology teaching in all years of medical school. Less than 15% reported having a form of pathology teaching weekly. 50% of the respondents reported being specifically examined in pathology in their end of year/final year examinations.

Discussion: The results of this survey support the views held by the RCPath and highlight potential areas for future development of medical curricula to enhance medical student perceptions and comprehension of pathology.





P56

Continuous Analysis of Histopathology Trainee Reports as a Means of Assessment

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Aim: We sought to determine the feasibility of a continuous assessment method by way of analysis of trainee reports. Secondarily, we hoped to evaluate the quality of reports to advocate or counter graded independent reporting by trainees. Method: Seven histopathology trainees were consented for the 6 month study. For each case they determined whether or not, if given the opportunity, they would report independently. All cases were thus categorised as either confident or non confident. Trainee reports were then compared to final issued report using a scoring system for macroscopy, microscopy examination and report formulation. Attention was given to report errors which would have led to an untoward clinical event. Other criteria included conveyance of appropriate certainty, use of relevant clinical comments and overall clarity. Data was compiled onto Microsoft Excel. Results: Entries focused on the microscopic examination whilst macroscopic and report formulation criteria were largely ignored. The two junior trainees were both confident of 73% of cases. Their overall accuracy rates were 87 and 75%. On average senior trainees (ST5s) confidence rate was 93% and overall accuracy ranged from 88 to 96%. The rate of major errors was 3 -4% for juniors and 1- 2% for seniors. For cases considered 'confident' the major error rate was below 0.8% for all trainees. Discussion: There was moderately low acceptability of data entry because it was deemed too time consuming. Fewer categories and criteria are likely to increase acceptability. Confidence and report accuracy increased with trainee experience. However, all trainees were adept at recognising cases where they were likely to make errors. Graded responsibility for independent reporting is supported by the low incidence of errors in confident cases.

P57

Do Medical Students Understand The Role of a Pathologist?

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The Royal College of Pathologists states that pathology is the hidden science at the heart of modern medicine, vital for the diagnosis and clinical management of disease. The pathology experience of medical students is declining and very few spend time in a pathology department. If they do not understand what the role of a pathologist is how can they be expected to be interested in a career in pathology? Method: To establish what medical students understand about the role of the pathologist, each medical student from 24 medical schools in England and Wales were sent an online survey. Results: 1126 students replied from 17 universities with even representation over years 1-5. Only 866 students agreed with the statement "a pathologist needs to be a medically qualified doctor" and 57 did not think a pathologist needed clinical knowledge and expertise to carry out their work. 99% and 92% thought pathologists' work involved post mortems and disease diagnosis respectively. 310 students thought pathologists did not need good communication skills and only 31% would consider a future career in pathology. Discussion: The findings show that the majority of medical students understand a pathologists' work involves diagnosis of disease and performing post mortems. However, the understanding of the attributes one must have to train and become a pathologist is clearly a problem and this may be as a result of lack of exposure to the specialty. Pathology is slowly increasing its profile and medical students should be our number one target audience.

P58

Histopathology Reporting of Screen-Detected, Versus Non-Screen-Detected, Colorectal Polyps

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BACKGROUND: The UK Bowel Cancer Screening Programme (BCSP) was introduced in 2007 to improve detection and management of early bowel cancers and pre-invasive lesions. Reporting guidelines, including a proforma, were published to encourage uniformity and ensure comparability of data between screening centres.
AIMS:
1. To assess the quality of histopathology reporting of colorectal polyps against the standard of current BCSP guidelines.
2. To compare the histological classification of screen-detected and non-screen-detected polyps.
METHOD: A retrospective case series (2009-2010) of 104 polypectomies and diagnostic biopsies was identified from computerised hospital records using standard SNOMED codes. Reports of 197 polyps were reviewed. Chi-square and t-test analyses were performed to evaluate differences between screen-detected and non-screen-detected cases.
RESULTS: Site and histological diagnosis were specified in all reports, but measurements were omitted from 17%. Screen-detected polyps (n=62) included a higher proportion of adenomas than non-screen-detected lesions (n=135; 72.3% v 62.2%; P<0.05) while the latter were more likely to be inflammatory (14.8% v 1.6%). All adenomas were classified as high- or low-grade in accordance with reporting guidelines. There were significant differences between reporting pathologists in the recording of core data, however, including completeness of excision and the presence or absence of stromal invasion (P<0.01).
CONCLUSIONS:
1. Under-reporting of core data occurred most often in benign lesions where it was unlikely to be of clinical significance. More complete recording, however, is needed to achieve compliance with BCSP guidelines.
2. The unexpected finding of a higher proportion of adenomas in the screened population may reflect small sample size.

P59

Examination of a Large Series of Temporal Artery Biopsies Shows That EVG Staining does not Contribute to the Diagnosis - a Follow on Study

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BACKGROUND: Elastic Van Gieson (EVG) stain continues to be used routinely to demonstrate disruption of the internal elastic lamina in clinically suspected giant cell arteritis (GCA). Recent reviews, however, have shown high variability for all features of GCA except the presence, extent and severity of mural inflammation. Previous audit of a small case series of positive biopsies confirmed that routine use of special stains in temporal artery biopsies does not increase diagnostic sensitivity, but this did not include review of EVG appearances in negative biopsies.
PURPOSE: To re-assess the contribution of EVG to the diagnosis of GCA, compared with standard haematoxylin and eosin (H&E)-stained sections alone.
METHODS: A retrospective case series of 525 temporal artery biopsies (Jan 1999-Oct 2009) was identified from computerised hospital records, yielding 105 biopsies positive, and 406 biopsies negative, for GCA. Positive biopsies were reviewed histologically against standard diagnostic criteria and comparable information obtained from reports of negative biopsies.
RESULTS: Review of H&E-stained sections demonstrated diagnostic features of GCA in 97.2% (n=102) of positive cases. Disruption or reduplication of the internal elastic lamina was apparent in 96.1% (n=101) of EVG-stained sections, but was also reported as present in nearly two-thirds of negative biopsies.
CONCLUSIONS: Results confirm that use of an EVG stain does not contribute to the recognition of diagnostic features in most cases of GCA and should therefore be limited to those with a strong clinical suspicion of GCA, but equivocal findings on H&E-stained sections. Inclusion of pre-biopsy treatment information on histopathology request forms would assist clinicopathological correlation in this group.

P60

Pitfalls to Using the Single WT1 C19 Antibody

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WT1 was first described as a tumour suppressor gene in Wilms tumour as it appeared to fit the classic two hit model of carcinogenesis. Lately however evidence has suggested that in some instances it appears to have a more oncogenic role and much work has centred around the overexpression of Wild type WT1. WT1 exists as several different isoforms which have slightly different molecular weights. The main WT1 isoforms have a molecular weight of 50-54kDa. In 1999 Scharnhorst et al found novel WT1 isoforms of 36-38kDa and 60-62kDa using the WT1 C19 Santa Cruz Biotechnology polyclonal antibody. However while investigating the oncogenic potential of WT1 using the same antibody we have discovered a larger protein band of 160 -260kDa present in several different cell lines ranging from breast (MCF7 & T47D), ovarian (A2780 & COV318) and renal carcinoma (RCC VHL & RCC VEC). Our first thought was a possible large WT1 complex as WT1 is known to dimerise or trimerise however as these bonds are non covalent they should be broken down during the protein extraction and western blot procedure so this can't be the case. It is therefore possible that it is a large molecule for example an immunoglobulin which is wrongly being picked up by this antibody. While this should not cause a problem in a western blot procedure it could cause problems in Immunohistochemistry or Immunoprecipitation reactions as these are non quantitative the anomalous band could cause a false positive and thus we need to be made aware of the potential for mistakes. It is therefore advisable to perform such investigations simultaneously with another WT1 antibody to corroborate any results.

P61

Qualifying an IHC Pharmacodynamic Biomarker - Androgen Receptor in Skin

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Quality assuring a pharmacodynamic biomarker can be divided into three steps for sufficient robustness to use for go/no go decisions in clinical development. The three steps are: Feasibility, Reproducibility, Positive control.
In clinical development proof of mechanism (PoM) biomarkers can evaluate if the drug hits the target to support go/no go decisions. Therapies targeting the androgen receptor (AR) e.g. bicalutamide, are important treatment options for prostate cancer patients. Collection of paired prostate biopsies to assess biomarker modulation before and on treatment can be challenging. Skin may be a surrogate tissue and potentially easier to collect.
This study evaluated if AR expression in skin could be quantified (feasibility) by immunohistochemistry (IHC) and to assess reproducibility, using prostate tumour as positive control. Twelve male healthy volunteers aged over 50 years were enrolled. Skin biopsies were collected at 3 time points on each of 2 days, one week apart. Biopsies were formalin fixed (24 hours), paraffin embedded. Sections were cut and stained with routine IHC method for AR (Dako). Stained slides were assessed by histopathologist and image analysis (Chromavision ACISII). AR expression was measured in the nuclear compartment of epidermis and sweat gland.
Overall variability of AR expression was high and dominated by the intra-subject (intra-day, inter-time) component (Coefficient of variability = 83%).
Conclusion: AR expression can be measured by IHC in skin. Whilst it may be feasible to assess AR expression for PoM in single centre healthy volunteer studies, given the biological variability it is unlikely to be useful in multicentre patient studies.

P62

A Rare Cause of Childhood Death: Clostridium Perfringens Sepsis in a Gastrostomy Fed Child with Cerebral Palsy

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A 13 year old boy with cerebral palsy had a 24 hour history of fever and diarrhoea prior to unexpected death at home. His body was received in the mortuary 24 hours after death, after appropriate refrigeration at the funeral home. The body was unusually foul-smelling and there was a bloated appearance to the body, with x-ray confirming extensive subcutaneous emphysema. Full post mortem examination revealed a gastrostomy in situ, repair of a previous atrial septal defect and a fundoplication. The stomach showed intramural gas with overlying mucosal splitting, and the small and large bowel were markedly distended by gas. The airways were erythematous and there were haemorrhagic changes in the lungs. The liver showed generalised necrotic softening with large gas bubbles. Histological examination revealed extensive growth of gram positive rods throughout most tissues, including the small bowel, with surrounding tissue necrosis and gas spaces. Microbiology revealed a pure growth of Clostridium perfringens from the spleen. Anaerobic cultures had not been performed on other samples, but the morphology of the gram positive rods seen throughout numerous organs is fully consistent with Clostridium perfringens. Death from Clostridium perfringens infection is rare in humans, and is usually associated with type C strains which cause necrotising enteritis. Unfortunately strain type was not available in this case, but the histology was consistent with necrotising enteritis. This child was gastrostomy fed with a sterile liquid feed which was found not to be contaminated on testing. Infection must therefore have originated from endogenous bowel bacteria. It is possible that some children with cerebral palsy have reduced splanchnic perfusion, and therefore mucosal ischaemic damage could be the initiating factor for infection in this case, in a situation analogous to Clostridial necrotising enterocolitis in premature neonates.

P63

Pulmonary Hyperplasia Secondary to Bronchial Atresia Causing Fetal Hydrops with Subsequent Intrapartum Death

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During the first pregnancy in a 26 year old mother, a fetal anomaly scan at 20 weeks' gestation showed significant abdominal ascites and an enlarged echo bright right lung with cardiac displacement. These findings were consistent with a type III congenital cystic adenomatoid malformation (CCAM). Labour was induced at 32 weeks gestation due to increasing polyhydramnios and maternal dyspnoea. Labour was complicated by abdominal dystocia and fetal paracentesis was performed, draining 800mls of fluid. A fetal bradycardia developed and unfortunately the baby was stillborn. Post mortem examination revealed atresia of the right lower lobe bronchus, and the right middle and lower lobes were markedly enlarged. The abdominal skin was lax due to previously drained ascites. Some haemorrhagic ascites remained, and the intestines and kidneys showed haemorrhagic venous infarction, attributed to impaired venous return due to the right lung mass. Histology of the lung mass showed a marked increase in alveolar and bronchiolar spaces. The features are typical of those traditionally described as type III CCAM, but in the context of bronchial atresia, are better described as pulmonary hyperplasia, similar to the changes seen in laryngeal atresia. This case is instructive in showing the profound haemodynamic disturbances that an intrathoracic fetal lesion causes, with fetal hydrops and haemorrhagic infarction of intra-abdominal organs due to impaired venous return. Current concepts of congenital cystic lesions that challenge Stocker's original classification are also discussed.

P64

Reporting of Lymphoma by Paediatric Pathologists: Five Year Experience of a Regional Children's Hospital

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BACKGROUND: Current national guidance indicates that a specialist haematopathologist should be involved in multidisciplinary team (MDT) review of all paediatric lymphomas. In our institution, like other children's hospitals in the UK, lymphomas are reported by paediatric pathologists, and discussed at the Paediatric Oncology MDT with paediatric oncologists. Expert haematopathology review is carried out only when the paediatric pathologist decides to refer the case. METHODS: An audit of 5 years' of haematopathology specimens reported by our department was carried out, to assess the workload in comparison to other paediatric tumours, and to look at referral patterns and concordance of diagnosis between paediatric and haematopathologists. RESULTS:From 2005-2009, 69 haematological malignancies and 254 benign conditions were reported. More cases of Hodgkin's lymphoma were received than Ewing's sarcoma/PNET, Wilms' tumours or rhabdomyosarcoma, with only neuroblastic tumours being reported in larger numbers. 7 of the malignant cases were referred for expert opinion (1 CHL with fragmented biopsy, 2 NLPHL, 1 B-LBL, 1 T-LBL, 2 FL), and in all cases the opinion agreed with that in the original report, except for one unusual case of FL with HL, where we deferred reporting to the expert opinion. Of 254 benign lymphoreticular conditions reported (115 lymph nodes, 124 tonsils, 14 adenoids and 1 skin), 7 cases were referred.

Tumour Type	Number of cases
Classical Hodgkin's Lymphoma (CHL)	25
Nodular Lymphocyte Predominant HL (NLPHL)	4
Precursor B Lymphoblastic Lymphoma (B-LBL)	3
Burkitt's Lymphoma (BL)	14
Diffuse Large B Cell Lymphoma (DLBCL)	2
Follicular Lymphoma (FL)	2
Precursor T Lymphoblastic Lymphoma (T-LBL)	8
Anaplastic Large Cell Lymphoma (ALCL)	5
Langherhan's Cell Histiocytosis (LCH)	3
Myeloid Sarcoma	2
Neuroblastic tumours	81
Wilms' Tumour	26
Rhabdomyosarcoma	10
Ewing's sarcoma/PNET	13

Reason for Referral	Haematopathology Opinion
2 cases unusual reactive pattern, rule out interfollicular Hodgkin's lymphoma	One EBV infection, one reactive
2 cases florid reactive pattern with progressive transformation of germinal centres	Agree
1 case clearly benign histology, referred due to strong clinical and radiological suspicion of malignancy	Agree benign
1 autopsy case, rule out immunodeficiency	Normal
1 case, immunohistochemistry suggestive of myeloid sarcoma in leukaemic patient	Reactive with mast cells and small vessels, no blasts

CONCLUSIONS: Paediatric lymphomas are among the commonest of paediatric malignancies. If appropriate and cautious referral to an expert is used, paediatric pathologists should be able to report lymphomas safely. Reporting by paediatric pathologists aids discussion of patients with paediatric oncologists, and turnaround times are far shorter than would be possible if samples were sent away to another centre.

P65

Congenital Cystic Malformation of the Oral Cavity: An Indication for Exit Procedure?

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Aims and Backgrounds: Congenital Cystic Malformations of the oral cavity are rare entities of fairly unknown etiology. Heterotopy or embryonal maldevelopment are discussed as possible causes. Many heterogeneous differential diagnoses, such as benign and malignant tumours have to be considered. Due to their potential of airway obstruction after birth a sudden clinical and/or surgical intervention may become necessary. Patient presentation: A male fetus showing a cystic tumour (about 1 cm in diameter) (3rd child of a 39 years old G6P2A3) by prenatal routine ultrasound scan was identified in the 19th week of gestation. During pregnancy the tumour showed a slightly increase of size (up to about 2.5 cm in diameter in the last trimester). A cesarean section with possible exit procedure was made in the 37th + 6 week of gestation because of the possibility of airway obstruction and respiratory distress. The newborn (3780 g body weight at birth) showed a 2.3 cm sized cystic tumour at the tip of the tongue. No disturbances in tongue movement nor respiratory distress were observed. Due to tumour size a decision for tumour enucleation was made. The specimen was examined histological showing a mucous filled connective tissue cyst with squamous and cylindric epithelium. Postoperative outcome was uneventful. Conclusion: Congenital tumours of the tongue are rare entities of unknown origin, which may be diagnosed accidentally by prenatal ultrasound or postnatal. Several differential diagnoses, such as other benign (e.g. ranula) or malignant tumours (malignant teratoma) are known, all of them may cause respirator problems. Dependent on size exit procedure can be necessary. Indication for postpartal surgical depends on tumour size and histology.

P66

Congenital Abdominal Cyst in a Female Patient – A Challenging Case

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Aims and Backgrounds: Congenital Abdominal Cysts are heterogeneous entities. Several differential diagnoses, e.g. ovarian cysts, hepatic cysts, intestinal duplications, cystic lymphatic malformations, have to be considered. In female fetuses ovarian cysts are the most frequent observed abdominal cysts. Treatment ranges from conservative procedure (wait and see) to fine needle aspiration and surgical intervention. Patient Presentation: A 29 years old G1P0 presented in the 36th + 3 week of gestation at the Department of Gynecology due to her female fetus showing sonographically a subhepatic cystic structure (52 x 31 x 45 mm) with sediment. No further anomalies were detected. The placenta was sonographically inconspicuous. Decision for cesarean section to avoid hemorrhage complication by compression of the cyst was made and performed without problems in the 38th + 4 week of gestation. The baby was observed postpartum and at the age of 11 weeks, the child, who appeared constantly diseased, developed an acute abdomen. By surgery a conglomerate tumour of the known cystic part (6 cm), which showed adhesions with inflammatory altered small bowel loops and the greater omentum, was found. The cyst was examined histological, presenting a hemorrhage infracted, non malignant soft tissue cyst with some calcifications, focal circular smooth muscle layers and a local peritonitis. No characteristic structures (not intestinal-, neither hepatic- nor ovarian-tissue) were found. Conclusion: Torqued ovarian cysts are observed more frequent before birth than postnatal. Sonographic verified sediment suggests a hemorrhage process or torsion. Complications associated with conservative treatment or fine needle aspiration have to be considered. Postpartal surgical treatment is, as well as indicating operative intervention on the cyst size (> 4 cm) discussed controversial, due to high tendencies of cyst regression in the further course

P67

Changing Patterns of Infant Death over the Last 100 Years: Autopsy Experience from a Specialist Children's Hospital

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Introduction: Paediatric autopsies have been performed at a specialist children's hospital in the United Kingdom since the 1800's, with the original hand written reports available for at least the last century. This study reviews the major findings from infant autopsies performed in 1909 and 2009 at this specialist centre.
Methods: Retrospective analysis of autopsies performed in the same unit 100 years apart. Infant deaths (excluding stillbirths or foetal cases) were identified and autopsy findings reviewed.
Results: In 1909, 357 autopsies were performed at the centre including 178 infants (50%), compared to 347 autopsies in 2009, including 128 infants (37%). The causes of death and patterns of disease identified at autopsy were significantly different between 1909 and 2009, namely, infection in 132 (74%; including 10 tuberculosis and diphtheria, and 19 meningitis) and 25 (20%; zero) respectively. Congenital heart disease was diagnosed in 4 (2%) and 9 (7%) infants with gastroenteritis in 19 (11%) and 1 (1%) and other gut anomalies in 17 (10%; including 8 congenital pyloric stenosis and 4 congenital absence of bile ducts) and 2 (2%) respectively. Only 1/178 (0.5%) was classified as unknown in 1909 compared to 48/128 (38%) in 2009.
Conclusion: During the last century, autopsy findings from a single paediatric centre demonstrate the changing pattern of fatal infant diseases, the earlier period associated with significantly more infectious deaths, especially due to meningitis, TB and diphtheria, and deaths from structural anomalies which are now surgically correctable, such as pyloric stenosis and congenital liver disease. Due to changes in referral patterns, disease epidemiology and pathology practice, unexplained infant death (SIDS) is now the commonest group, being almost never encountered, or reported, in 1909.

P68

Autopsy Findings in Sudden Unexpected Early Neonatal Death: Results from a Specialist Centre

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Introduction: Sudden unexpected early neonatal death (SUEND) in the first week of life shares some features with sudden unexpected death in infancy (SUDI; 7-365 days) but is not usually included in SUDI statistics. This study reviews findings of >100 consecutive SUEND autopsies performed at a specialist centre.
Methods: Data from >2,750 consecutively performed paediatric autopsies performed by specialist paediatric pathologists at one centre according to a standard protocol were retrospectively recorded into an autopsy database. SUEND cases were identified and autopsy findings reviewed.
Results: Of 2,762 paediatric autopsies, 299 were deaths in the first week of life, including 101 (34%) presenting as SUEND of an apparently clinically well infant. 64 (64%) were explained following autopsy, whilst 36% remained unexplained. 10 (27%) of the unexplained deaths were associated with co-sleeping with an adult. Of the explained deaths, 17 (27%) were infection-related, 11 (18%) due to metabolic causes and 15 (24%) undiagnosed congenital abnormalities, mainly congenital heart disease.
Conclusion: Two-thirds of SUEND are explained following autopsy, compared with around one third of SUDI, but one third remains unexplained, analogous to SIDS in older infants. In this group of explained deaths, infection is the commonest cause of death, followed by unsuspected congenital abnormalities and metabolic disease.

P69

Autopsy Findings in 885 Consecutive Sudden Unexpected Deaths in Infancy Examined at a Single Specialist Paediatric Centre

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Introduction: Sudden unexpected death in infancy (SUDI) remains the commonest presentation of post-neonatal infant death, with various hypotheses regarding the cause and mechanism of death. This study reviews the findings from a consecutive series of SUDI autopsies performed in a single specialist centre over a 14 year period (1996 to 2009).
Methods: Data from >2,750 consecutively performed paediatric autopsies performed by specialist paediatric pathologists at one centre according to a standard protocol were retrospectively recorded into an autopsy database. SUDI cases were identified and autopsy findings reviewed.
Results: Of 2,762 consecutive autopsies, 1,228 were infants between the ages of 7- 365 days. Of these, 885 presented as SUDI. 334 cases (38%) were explained following autopsy leaving 551 unexplained cases, representing SIDS / unexplained SUDI. Of 334 explained deaths, 188 (56%) were infection-related, 35 due to cardiovascular system abnormalities (4%; including 32 congenital heart disease). There were 33 cases (4%) with features of non-accidental / inflicted injury as the cause of death. Of 458 unexplained SUDI in whom death was recorded as during normal sleep, 237 infants were co-sleeping (52%), compared to 14% co-sleeping in the explained death group (19/138).
Conclusion: In a large series of SUDI autopsies at a specialist centre, including ancillary investigations, around 60% of deaths remain unexplained, with more than half of these being co-sleeping associated. Infection represents the commonest currently detectable aetiology of explained SUDI cases.

P70

Autopsy Findings in Paediatric Sickle Cell Disease

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Introduction: Sickle Cell Disease (SCD) is a common single gene disorder, with significant morbidity and mortality despite improvements in healthcare. Death in childhood from SCD is rare. This study reviews findings from paediatric autopsies in a single specialist centre of cases with SCD.
Methods: Data from >2,750 consecutively performed paediatric autopsies at one centre were retrospectively recorded into an autopsy database. Deaths where the patient was known to have SCD were identified and autopsy findings reviewed.
Results: Of 2,762 paediatric post-mortem examinations, only 11 (0.4%) patients were identified with SCD. At time of presentation, one was not known to have SCD: being diagnosed after collapse. One patient had been diagnosed immediately prior to collapse. Three patients suffered from previous sickle crises requiring hospital admission. One patient presented with a fatal first crisis. Seven were referred as sudden unexpected deaths. 8 (73%) patients showed extensive sickling, with or without haemorrhage, in at least one site. Of all cases, 6 (55%) died from direct consequences of SCD (splenic sequestration, sickle cell crises, acute chest syndrome, acute cerebral infarct). Three died from infections, including viral gastroenteritis and pneumococcal sepsis, and one case, the youngest death in the group (22 days of age), did not show sickling on histology but died directly from bronchopneumonia. One died from a ruptured aneurysm of the posterior cerebral artery, with subarachnoid haemorrhage, a known association with SCD.
Conclusion: The autopsy revealed expected and unexpected findings in these cases with SCD. Around half died due to sickling complications, the others from related or unrelated causes. In 20%, the death was the initial presentation of SCD. These findings emphasise the need for aggressive management of patients with SCD in order to reduce mortality and morbidity.

P71

Autopsy Findings in Paediatric Immunodeficiency: Cases from a Specialist Paediatric Centre

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Introduction: The diagnosis of primary immunodeficiency diseases has improved over the past 10 years with advancing medical knowledge, in particular with regards to the genetics of such diseases, most presenting clinically with recurrent infection of systemic disease. This study reviews autopsy findings of such cases.
Methods: Data from >2,750 consecutively performed paediatric autopsies performed by specialist paediatric pathologists at one centre according to a standard protocol were retrospectively recorded into an autopsy database. Cases where a diagnosis of primary immunodeficiency had been made were identified and the autopsy findings reviewed.
Results: Of 2,762 consecutive paediatric autopsies, 17 (0.6%) cases were identified with a diagnosis of immunodeficiency, including one case of common variable immunodeficiency (CVID), five of severe combined immunodeficiency (SCID), five of presumed primary undetermined immunodeficiency (based upon clinical and autopsy findings) and six cases with DiGeorge syndrome. 2 presented as sudden unexpected death in infancy, all others cases being clinically unwell and undergoing investigations prior to death, including all six DiGeorge syndrome cases with congenital cardiac disease. 7 deaths (41%) were associated with atypical infections (including Pseudomonas, Pneumocystis jiroveci, and Cytomegalovirus), 6 were due to complications of congenital heart disease and the remainder due to other causes.
Conclusion: Primary immunodeficiencies are rarely seen in paediatric autopsy practice, most presenting clinically prior to death, but occasionally presenting as sudden unexpected death in infancy, the diagnosis being made at autopsy. Of the non DiGeorge cases without congenital heart disease, two-thirds were deaths associated with atypical infection.

P72

Autopsy Investigations Suggestive of Metabolic Causes of Death

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Introduction: Metabolic disease can be challenging to detect clinically and may present as Sudden Unexpected Death in Infancy, posing difficulty with autopsy interpretation of ancillary investigations.
Methods: Data from >2,750 consecutively performed paediatric autopsies performed by specialist paediatric pathologists at one centre according to a standard protocol were retrospectively recorded into an autopsy database. Infant deaths with Oil-Red-O staining of frozen sections and blood or bile Tandem Mass Spectrometry (TMS) Guthrie results were identified and autopsy findings were reviewed.
Results: Of 2,762 consecutive paediatric autopsies, 870 were infants in whom Oil-Red-O (ORO) staining was performed on frozen samples of heart, muscle, kidney and liver. 93 (11%) showed significantly increased fat in at least two tissues. TMS results demonstrated 13 metabolic diagnoses (mainly fatty acid oxidation disorders). Of 22 cases of abnormal fat staining in only 2 tissues, 1 had a positive TMS result (5%); of 24 with abnormal staining in 3 tissues, 4 (17%) had positive TMS results, and of 47 with abnormal staining in all 4 organs, 8 (17%) had positive TMS results. Bile Guthrie results were in accordance with Blood Guthrie results in all cases. Only one autopsy with equivocal staining on ORO had a positive TMS result.
Conclusion: Ancillary testing with Oil-Red-O staining for metabolic disease is a cheap and effective screen for most metabolic disorders presenting as infant death and identifies cases at increased risk of metabolic disease in whom formal TMS testing should be performed.

P73

Metabolic Causes of Neonatal and Infant Death: Autopsy Findings from a Specialist Paediatric Centre

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Introduction: Metabolic diseases can present as sudden unexpected infant death and can be challenging to detect in the neonatal and infant period. This study reviews cases of infant death due to metabolic disease examined at autopsy at a specialist centre.
Methods: Data from >2,750 consecutively performed paediatric autopsies performed by specialist paediatric pathologists at one centre according to a standard protocol were retrospectively recorded into an autopsy database. Metabolic causes of death were identified and the autopsy findings reviewed.
Results: Of 2,762 consecutive paediatric autopsies, 1,527 represented liveborn infants (<366 days). In total, 59 (4%) cases were identified where either by pre-mortem findings (including clinical history), autopsy findings or ancillary testing were either suspicious for, or diagnostic of an underlying metabolic disorder. 38 (64%) presented as sudden unexpected death with 18 in the first week of life, and 20 aged 7-365 days. 21 presented with preceding clinical features of disease. Of the 59 cases, 31 (53%) occurred within the neonatal period. Causes included mitochondrial defects, fatty acid oxidation disorders and other inborn errors of metabolism.
Conclusion: Metabolic disease may present as neonatal or infant death, more than half of fatal cases presenting as sudden unexpected death, the diagnosis being suggested at autopsy. Selection of appropriate tissue sampling during the autopsy procedure and appropriate ancillary investigations can help confirm the diagnosis and be of help with genetic counselling for parents with future siblings.

P74

Autopsy Findings in Neonatal Deaths; >500 Consecutive Cases from A Specialist Paediatric Centre

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Introduction: Neonatal autopsy rates have fallen in recent years, and only highly preselected cases are referred for autopsy. This study reviews findings from a series of >560 consecutive neonatal autopsies performed in a single specialist centre. Due to the tertiary nature of the centre, and the bias in clinical history of cases referred for autopsy, the cases undergoing autopsy are not representative of unselected neonatal deaths.
Methods: Data from >2,750 consecutively performed paediatric autopsies performed by specialist paediatric pathologists at one centre according to a standard protocol were retrospectively recorded into an autopsy database. Neonatal deaths were identified and autopsy findings reviewed. Results: Of 2,762 consecutive paediatric autopsies, 564 represented deaths of liveborn infants in the neonatal period (<28 days), of which 245 (44%) presented as sudden unexpected deaths, either in the first week of life (sudden unexpected early neonatal death (SUEND)) or at 7-28 days (sudden unexpected death in infancy (SUDI)). Overall, 411 deaths (73%) were explained following autopsy; 131 (32%) being due to congenital abnormalities (including 102 congenital heart disease), 85 (21%) were infection-related deaths (including 66 bacterial, 18 viral and 1 fungal) with respiratory tract infection being the commonest infectious process.
Conclusion: The majority of neonatal deaths referred for autopsy to a tertiary centre are explained following post-mortem examination. Congenital abnormalities, especially congenital cardiac disease, and infection, represent the commonest aetiologies of death. Almost half of referred cases represent SUEND or SUDI, with about half of these remaining unexplained. Deaths due to common clinically recognisable neonatal complications such as prematurity-related disorders (respiratory distress syndrome, necrotising enterocolitis), are now rarely referred for post-mortem examination.

P75

Difficulty of Interpretation of Post-Mortem Microbiology Results in Unexpected Infant Deaths: Evidence from a Multidisciplinary Survey

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Introduction: Interpretation of post-mortem microbiology results in investigation of sudden unexpected death in infancy (SUDI) remains difficult. A cross-specialty questionnaire survey was performed to assess interpretive differences and difficulties.
Methods: 109 consultant specialists involved in infant death management were contacted (including Paediatric Pathology, Histopathology, Microbiology, Paediatric Infectious Diseases and Paediatrics/Child Protection) with information on 5 standardised SUDI cases differing only in their PM microbiology findings from blood, spleen and lung. Respondents classified each case regarding likely cause of death into 4 categories (definite bacterial infection through post-mortem contamination). Findings were chosen to represent definite infection/non-infection cases and more difficult cases.
Results: 63 specialists responded (57%). There were no scenarios in which all specialists agreed. Concordance rates varied; Case 1-76% agreed definite bacterial infection and 20% probable bacterial infection. Case 2, 70% agreed probable bacterial infection. Case 3, 58% agreed post-mortem overgrowth/contamination. Case 4, (*Staphylococcus aureus* positive cultures) caused the most disagreement with 50% suggesting probable bacterial infection. Case 5, 82% agreed post-mortem contamination, including all paediatric pathologists. There were no other significant differences in responses between specialties.
Conclusion: Interpretation of PM microbiology results in SUDI is difficult with no agreement of interpretation by specialists, discordant opinions regarding the significance of the same findings being seen both within and across specialties. The interpretation of *S. aureus* positive cultures is particularly challenging. Paediatric pathologists are more likely than paediatricians to attribute findings to PM overgrowth. Further research to aid correct interpretation is urgently required.

P76

Clinicopathological Features of Paediatric Deaths due to Cardiomyopathy: An Autopsy Series

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Introduction: Cardiomyopathy is an uncommon but potentially fatal condition which encompasses many types with a range of aetiologies and modes of presentation. This study reviews clinical and pathological features of children presenting with cardiomyopathy-associated death at a single specialist centre.
Methods: A retrospective review was carried out of 1,516 paediatric autopsies carried out at the centre from 1996 to 2005 inclusive, to identify cases in whom a diagnosis of cardiomyopathy was made at autopsy.
Results: 32 potential cardiomyopathy deaths were identified, 13 of which were excluded from further analysis (11 post-cardiac transplant deaths; 2 myocarditis). Of 19 cases included, the final diagnosis was dilated cardiomyopathy in 11 (58%), hypertrophic cardiomyopathy in three (16%), arrhythmogenic right ventricular cardiomyopathy in two (11%), metabolic cardiomyopathy in two (11%), and one case of histiocytoid cardiomyopathy. The age at death was 10 days to 15 years (median 18 months), with nine (47%) infants under one year of age, five (26%) aged 1-4 years, and six (32%) older children. Cardiomyopathy-associated deaths represented 0.9% of all 965 infant autopsies, 1.9% of 261 childhood deaths at 1-4 years of age, and 4.1% of paediatric autopsies aged 10-18 years. The majority (16; 85%) manifested some symptoms prior to death, whilst 3 (15%) presented as apparently sudden unexpected death without prodromal features. In more than half (10; 53%) of symptomatic deaths the diagnosis of cardiomyopathy was only established at autopsy.
Conclusion: Cardiomyopathy is an uncommon but recognisable cause of death, representing about 1% of paediatric deaths referred for autopsy to a single specialist centre over a 10-year period.

P77

Co-Sleeping and Sudden Unexpected Deaths in Infancy (SUDI): Autopsy Series from a Single Specialist Centre

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Introduction: It is suggested that co-sleeping increases risk of sudden unexpected death in infancy (SUDI), particularly when associated with maternal smoking, or parental alcohol or drug use. This study determines the prevalence of co-sleeping in SUDI deaths referred for autopsy to a single specialist centre.
Methods: Retrospective analysis of >1,500 consecutively performed post-mortem examinations at a single centre over a 10-year period (1996-2005). SUDI was defined as death of an infant 7-365 days that was sudden and unexpected; deaths were categorised into explained (cause of death was found at autopsy) or unexplained SUDI.
Results: Of 546 SUDI, 314 infants died during sleep in whom sleeping arrangements were well documented; of these, 174 (55%) were co-sleeping-associated deaths. More than half (59%) of unexplained SUDI were associated with co-sleeping, usually with one or both parents, compared to only 44% of explained SUDI (difference 14.4%, 95% CI 1.0-27.2%, p=0.03). In both groups, co-sleeping-associated deaths were more common in younger infants; however, co-sleeping-associated deaths were significantly more common in unexplained SUDI than explained SUDI only in the first six months of life (66% unexplained SUDI vs. 47% explained SUDI; difference 18.6%, 95% CI 3.5-33.2%, p=0.01), but not in older infants aged 6 to 12 months (27% vs. 35%; difference 8.6%, 95% CI -15.1-35.3%, p=0.38). Overall, 32 (18%) co-slept on a sofa.
Conclusion: Co-sleeping is common, associated with more than half of SUDI, and is especially associated with unexplained SUDI in infants aged less than six months, suggesting that co-sleeping may be related to the pathogenesis of sudden death in younger infants.

P78

Herpes Simplex Viral Infections in Paediatric Deaths

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Introduction: Herpes simplex virus (HSV) infections are a recognised cause of death in infants, but the frequency and age distribution of HSV-related deaths remain uncertain. This study reviewed paediatric deaths referred to autopsy associated with HSV infection.
Methods: A retrospective review of >1,500 consecutively performed autopsies at a single specialist paediatric centre over a 10-year period (1996-2005). Findings of deaths in which HSV was demonstrated at autopsy were reviewed.
Results: Of 1,372 paediatric autopsies over a 10-year period, in 11 (0.8%), HSV was identified at autopsy, either on virological testing, or histology and immunohistochemistry. One was a 6-year old girl who died suddenly with necrotising HSV encephalitis, with no apparent underlying immunodeficiency; and one was a sudden unexpected co-sleeping-associated death of a 7-month old boy, in whom no cause of death could be determined at autopsy, with no histological evidence of infection, but in whom HSV was detected on PCR of the spleen. All other cases were neonatal deaths (7-15 days, median 14 days); one died from myocarditis associated with HSV and enteroviral infection, the other eight, representing 2% of 366 neonatal deaths (<28 days), died from disseminated HSV infection, all of which demonstrated liver involvement in addition to other organs. The diagnosis was based on a combination of histological, immunohistochemical and virological investigations.
Conclusion: Disseminated HSV infection is a rare cause of death at autopsy, accounting for 0.6% of all deaths (2% of neonatal deaths), and is largely restricted to the neonatal period, likely representing congenitally acquired infections.

P79

Staphylococcal Toxins in Sudden Unexpected Death in Infancy (SUDI)

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Introduction: Two-thirds of sudden unexpected deaths in infancy (SUDI, aged 7-365 days) remain unexplained following autopsy. It has been postulated that some deaths may be caused by toxigenic Staphylococcus aureus (SA). This study compared prevalence of toxigenic SA in unexplained and explained SUDI (those in whom a cause of death is determined).
Methods: A retrospective review of SUDI autopsies as part of a larger review of >1,500 paediatric autopsies over a 10-year period was performed. SUDI cases were categorised as unexplained, explained with histological evidence of infection (bacterial infection group) or explained due to non-infective causes. Toxin gene profiling was carried out by PCR as part of routine investigation in cases with positive SA cultures.
Results: Of 507 SUDI, bacteriological investigations were performed in 470. SA was isolated on post-mortem cultures in 173 (37%). There were significantly more cases with SA identified in unexplained SUDI (40%) than in the non-infective SUDI group (21%; difference 19.0%, 95% CI 5.4-29.3%, p=0.006), but no significant difference in prevalence between the former and the bacterial infection group (33%; difference 7.0%, 95% CI -7.4-19.4%, p=0.30). 46% of cases with SA underwent testing for the panel of staphylococcal toxin genes (including SEA to SAE, SAG to SAJ, TSST-1, and exfoliative toxins A and B). There were more slightly cases with at least one toxigenic strain of SA in the unexplained SUDI (81%) and bacterial infection groups (77%) than in the non-infection group (63%), but these differences were not statistically significant (Fisher exact test p=0.44).
Conclusion: Carriage of toxigenic strains of SA is more common in unexplained SUDI, further supporting the hypothesis that a subset of SUDI may be related to the production of bacterial toxins, but toxin gene testing does not demonstrate differences in frequencies between groups.

P80

Cardiac Causes of Sudden Unexpected Deaths in Infancy and Childhood: Autopsy Series

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Introduction: Cardiac disorders are a recognised cause of sudden unexpected death (SUD) in infancy and childhood. This study examines the frequency and characteristics of sudden cardiac deaths referred for autopsy to a single specialist centre.
Methods: From a review of autopsies carried out in a single paediatric centre over a 10-year period, sudden unexpected cardiac deaths were identified; those in which the underlying cardiac abnormality was first detected at autopsy. SUDs in children with pre-existing cardiac pathology diagnosed during life were excluded.
Results: Of 1,372 paediatric autopsies (0-18 years), 855 (62%) were sudden and unexpected deaths. 416 (49%) deaths were explained by the autopsy findings, of which 55 (13%; 6% of all SUDs) were due to previously unsuspected cardiac pathology, accounting for 34% of explained sudden unexpected early neonatal deaths (<7 days), 13% of explained SUD in infancy (7-365 days), and 6% of explained SUD in children aged 1-4 years, 10% aged 5-9 years, 27% aged 10-14 years, and 29% aged ≥15 years. Causes included structural congenital heart disease (CHD) in 23 (42%), myocarditis in 20 (36%), cardiomyopathy in 8 (15%) and other in four (7%; Kawasaki disease, coronary artery atheroma with homozygous familial hypercholesterolaemia, infarction of the atrioventricular node, and dilated (primary) endocardial fibroelastosis). Whilst myocarditis occurred in all ages, death due to previously undiagnosed CHD was limited to children <4 years of age, and cardiomyopathy to infants (<365 days) and older children (≥10 years).
Conclusion: Previously undiagnosed cardiac pathology accounts for the cause of death in around 6% of all SUDs in infancy and childhood. The spectrum of cardiac abnormalities varies and is related to the age of the child.

P81

Variation and Uncertainties in the Classification of Sudden Unexpected Infant Deaths amongst Paediatric Pathologists in the UK: Findings of a National DELPHI Study

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Introduction: Paediatric Pathologists use diverse terminologies to classify sudden unexpected infant deaths (unexplained, unascertained, sudden unexpected death in infancy/SUDI, and sudden infant death syndrome/SIDS). This study used the DELPHI method to investigate views of UK paediatric pathologists on their use of these terms in order to identify areas of consensus and disagreement.
Methods: A standard DELPHI approach was used, with three email rounds. In the final one, once views had been collated, participants scored statements using a modified Likert scale (0-9). Scores were analysed using non-parametric statistics; statements with median scores ≤3 or ≥7 were considered to have reached consensus agreement.
Results: 25 of 36 UK Paediatric Pathologists who were approached contributed to the final round. There was consensus that "SIDS" be used for otherwise unexplained deaths occurring during sleep; infancy was defined as up to one year of age but there was no consensus regarding the lower age limit for SIDS. SUDI was used for deaths with atypical but non-suspicious circumstances of death (e.g. co-sleeping-associated deaths), whilst unascertained was used for unexplained deaths in whom findings were suspicious of a possible non-natural cause.
Conclusion: There remains significant lack of agreement in terminology used by UK Paediatric Pathologists, suggesting that an acceptable alternative term be identified to classify infant deaths which remain unexplained following autopsy with no suspicious features but not necessarily typical for SIDS; we propose that "unexplained SUDI" followed by a comment may represent the most factually correct compromise, but this requires further evaluation.

P82

Pneumocystis (Carinii) Jiroveci Identified at Autopsy: Findings from a Specialist Paediatric Centre

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Introduction: *Pneumocystis Jiroveci* (*PCJ*) is a unicellular fungus usually recognised as an opportunistic infection in immunosuppressed individuals. This study reviews autopsy findings from a single specialist paediatric centre in which *PCJ* has been identified.
Methods: Data from >2,750 consecutively performed paediatric autopsies performed by specialist paediatric pathologists at one centre according to a standard protocol were retrospectively recorded into an autopsy database. Cases in which *PCJ* were detected on immunofluorescence were identified and the autopsy findings reviewed.
Results: Of 2,762 consecutive paediatric autopsies, lung microbiology was performed in 1,057 liveborn infants. 29 cases in total had *PCJ* detected by immunofluorescence, all but one of which were infants (65-339 days of age), the prevalence in infant deaths being 28/1057 (2.6%). 23 of the 28 infants presented as sudden unexpected deaths. Histologically, *PCJ* was identified in lung tissue sections in 13 cases (46%) and apart from two cases, there was associated inflammatory change. In total, of these 29 cases, 18 showed an inflammatory process ranging from a mild peribronchial mononuclear infiltrate to a severe acute pneumonia. In 7 cases, no definite other cause of death was found, in another 7, co-morbidities were present as the cause of death including congenital defects and neurological insults. In the remaining 15, there was evidence of pneumonia (7), pneumonitis (5) and systemic sepsis (3). There was associated co-infection in 9, including Cytomegalovirus, *Streptococcus pneumoniae* and Group B *Streptococcus*. In 4 cases, underlying immunodeficiency states were diagnosed.
Conclusion: Colonisation by *PCJ* appears to occur in the first year of life. In 7 of 1057 infants, *PCJ* may be identified in lung sections with no histological pneumonitis and no other cause of death. The significance of this finding remains uncertain.

P83

Mesocolic Excision with Central Vascular Ligation not Extended Longitudinal Excision may be the Key to Improved Survival in Colon Cancer: Lessons from Japan

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Over recent years there have been increasing calls to recognise the importance of high quality colon cancer surgery after years of emphasis on total mesorectal excision. We have previously shown that mesocolic plane surgery is associated with better survival and that complete mesocolic excision with central vascular ligation (CME with CVL) results in an oncologically superior specimen. Surgeons from Japan report similar outcomes to CME with CVL surgeons. We aimed to further investigate Japanese surgical principles and determine which factors may be most important.

We received clinicopathological data and fresh specimen photographs from 77 cases of primary colon cancer resected in two centres in Japan. The plane of surgery was assessed from the photographs and tissue morphometry was performed.

71% of specimens were resected in the mesocolic plane and none showed incisions down to the muscularis propria. The median length of bowel removed was 150mm (IQR 132 to 201mm) and the median distance from the tumour to the vascular tie (VT) was 110mm (IQR 97 to 137mm). The length of bowel was the same for right and left sided resections, however, right sided resections had a shorter distance to the VT (101 vs. 123mm, p=0.038). There was a positive correlation between body mass index (BMI) and the area of mesentery removed (r=0.467, p<0.0001). The median lymph node yield was 20 (IQR 13 to 24) which was not significantly correlated to the amount of tissue removed or BMI.

We have shown that Japanese surgeons predominantly resect colon cancer in embryological tissue planes with a high VT in a similar fashion to CME with CVL surgeons. However, they remove a shorter length of colon resulting in a lower lymph node yield. With impressive survivals reported, including in stage III disease, the principles of operating in the correct plane with a high vascular tie appears to be more important than the actual length of bowel removed.

P84

Measuring Colorectal Polyps Endoscopically and Histologically: Does it Matter?

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Colorectal polyps must be carefully examined to identify malignancy and determine the risk of developing further neoplastic lesions. An adenomatous polyp measuring 10mm or more in maximum size will trigger follow up by the NHS Bowel Cancer Screening Programme. It is important to determine the frequency of inclusion of size in endoscopy and histopathology reports, and the degree of correlation between the modalities.

All endoscopic polypectomies performed in a 3 month period were identified, the endoscopy and histopathology reports collected and the relevant data extracted. Additional measurements of the maximum polyp size were performed on the glass slides using a ruler and a breast screening magnifier device.

222 patients underwent endoscopic polypectomy yielding 393 lesions including 264 adenomas. Of the adenomas, 89% showed low grade dysplasia, 9% high grade and 1% invasive adenocarcinoma. For all polyps, endoscopic size was reported in 83% (mean 6.7mm) and histological size in 15% (mean 12.2mm). For the adenomas, endoscopic size was reported in 88% (mean 7.8mm) and histological size in 21% (mean 12.6mm). Of the 268 polyps removed intact, endoscopy appeared to overestimate the size in 68% and underestimate in 16% when compared to the ruler measurement. For 24 cases, the endoscopy size fell on different sides of the 10mm cut-off to the ruler size (17 over and 7 below). The magnifier was strongly correlated to the ruler measurement (R=0.947, p<0.0001) but appeared easier to use.

Pathologists are poor at recording polyp size, even in adenomas, resulting in the intensity of follow up being determined by endoscopic size for the majority of lesions. If a ruler measurement on the glass slide is considered as the gold standard, the use of endoscopic measurements will overestimate the size of the majority of polyps resulting in a significant number of patients unnecessarily entering intensive follow up programmes.

P85

Investigating the Factors Linked to Histopathological Recovery in Coeliac Disease

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Coeliac disease is a relatively common condition which is usually managed by placing patients on a gluten free diet. Follow up biopsies to confirm histological recovery are controversial with a considerable variation in practice observed. We aimed to determine the length of time to histopathological recovery in a group of coeliac disease patients and assess its associations with clinicopathological data.

All patients attending a specialist coeliac disease clinic prior to March 2009 were entered onto a database which recorded various clinicopathological data. The histopathology reports for all duodenal biopsies were reviewed and each biopsy was given a histopathological disease score based on a modified Marsh grade.

284 patients underwent index and at least one subsequent biopsy. 227 (80%) showed histopathological improvement and 100 (35%) returned to normal (median recovery time 1.9 years, IQR 1.0-4.8 years). Patients with less severe disease at diagnosis were more likely to show a better response (r=0.281, p<0.0001). Older patients demonstrated a shorter time to histopathological recovery (r=-0.200, p=0.001). Compliance with a gluten free diet was correlated with the best follow up biopsy score (r=-0.134, p=0.040) and degree of histological recovery (r=0.161, p=0.014).

Current guidelines for the timing of repeat biopsy after commencing a gluten free diet are unclear, although 4-6 months has been recommended. This study shows that time to histological recovery is longer than traditionally thought and may need to take into account the patient's age at diagnosis, the initial disease score and the level of compliance with a gluten free diet.

P86

No Relationship Between the Expression Patterns of CDH1, CTNNB1, BCL2 and EGFR in Gastric Cancer

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Aberrant E-cadherin (CDH1) expression is a common finding in gastric cancer (GC). It has been suggested that CDH1 is involved in regulating the expression of proteins involved in EGFR signalling, WNT signalling and apoptosis. Studies in the past have usually investigated CDH1 expression in GC in isolation. We aimed to assess the concurrent expression of CDH1, beta-catenin (CTNNB1), BCL2 and EGFR and its relationship to clinicopathological data and patient survival.

Tissue microarrays were constructed from 110 GC and protein expression was studied by immunohistochemistry (IHC). IHC staining was categorised as positive/negative (BCL2), abnormal/normal (CDH1), negative/membranous/nuclear (CTNNB1) and negative/weaker/equal/stronger than matched normal mucosa (EGFR).

11% GC were BCL2 positive, 41% GC showed abnormal CDH1 expression, 69% GC were CTNNB1 negative. 18%GC were EGFR negative, 59% GC showed the same or lower EGFR staining intensity than matched normal. 24% GC showed EGFR overexpression compared to matched normal mucosa. No relationship was found between the expression of CDH1, BCL2, CTNNB1 and EGFR on bivariate correlation analysis. Loss of CTNNB1 was more frequent in high pT (p=0.004). Loss of EGFR was related to poor patient survival whilst patients with normal-like EGFR expression survived longest (p=0.026, univariate analysis).

Disappointingly, bivariate correlation analysis did not show the hypothesised related expression pattern between CDH1 and its suggested downstream targets. Hierarchical clusteranalysis may be required to show such a relationship. However, this is the first study to demonstrate that GC patients with complete loss of EGFR have a worse prognosis than those with EGFR overexpression. As EGFR is part of a complex regulatory network, further studies are warranted to analyse the effects of loss of EGFR protein expression in GC.

P87

Quantitative and Qualitative Relationship of Tumour Cells and Vessels in Gastric Cancer – Identification of Three Distinct Vascular Patterns

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Anticancer drug resistance is usually considered to be related to molecular changes of the tumour cell thus disregarding the potential influence of the tumour microenvironment. However, we think that drug response may depend on tumour cell density, overall tumour vessel density and spatial relationship between vessels and tumour cells ('vascular pattern'). No data is available on the relationship of tumour cells and vessels in gastric cancer (GC). We undertook a pilot study to measure overall tumour vessel density (TuVD), tumour cell density (TuCD) and determine the vascular pattern (VP).
HE and CD34 stained sections of 52 GC were scanned and quantitatively assessed by point counting using 2000 points for TuVD and 300 for TuCD. VP was determined by visual inspection at low magnification. All slides were scored by 2 observers.
A wide variation of TuVD (median: 7%, range: 2 to 28%) and TuCD (median: 34%, range 13 to 88%) was noted. High TuCD was related to low TuVD (p=0.024). Three major vascular patterns were observed (A) vessels evenly distributed, compressed and directly adjacent to small groups of tumour cells (30% of GC) (B) vessels randomly distributed and directly adjacent to tumour cells (40% of GC) and (C) vessels randomly distributed and separated by clearly visible stroma from tumour cells (30% of GC). VP-B was more frequent in diffuse type GC (p= 0.037). No relationship with pT, pN, grade or survival was found in this pilot study.
This is the first study that quantified both, overall tumour vessel density and tumour cell density demonstrating that there is a relationship between the two, a finding which may be important for anticancer drug resistance. Furthermore, three qualitative different vascular patterns were identified in GC for the first time. These could be related to different molecular phenotypes of tumour cells. Further studies in larger series are necessary for confirmation and validation.

P88

Loss of MLH1 and MSH2 Expression Varies Significantly between Sporadic Cancers of the Gastrointestinal Tract – No Relationship With Survival in 1027 Cases

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Mismatch repair proteins, MLH1 and MSH2, play a crucial a role in DNA repair. Loss of MLH1/MSH2 expression results in microsatellite instability, one of the two major forms of genetic instability in cancer. The incidence of oesophageal (OeC), gastric (GC), small bowel (SBC) and colorectal (CRC) cancers varies substantially from 1.9/100,000 per year in SBC to 49.1/100,000 per year in CRC. The aim of our study was to investigate the frequency of loss of MLH1/MSH2 expression and its relationship to tumour location, clinicopathological data and patient survival.
Tissue microarrays and immunohistochemistry were used to assess and compare frequency of loss of MLH1/MSH2 protein expression in 1027 cases of gastrointestinal (GI) tract cancers: 165 OeC, 372 GC, 160 SBC and 330 CRC. Relationship to clinicopathological data and survival was established for each site.
Loss of MLH1/MSH2 expression was significantly different (all $p<0.05$) between different GI tumour sites and was observed for MLH1 in 0.6% OeC, 8% GC, 13% SBC, 12% CRC; and for MHS2 in 0.6% OeC, 4% GC, 8% SBC and 3% CRC. Loss of MLH1/MSH2 was more frequent in right than left sided CRC. No relationship of loss of MLH1/MSH2 and pT, pN, grade or survival was seen in any of the tumour sites.
This is the first study comparing MLH1/MSH2 expression in a large series of cancers of the upper and lower GI tract. Whilst the frequency of loss of MLH1/MSH2 varies significantly throughout the GI tract, loss of MLH1/MSH2 is not a prognostic factor in any of the GI cancers. SBC has the highest frequency of loss of MLH1/MSH2 indicating that microsatellite instability may play a much more important role in SBC carcinogenesis than in OeC and GC. Whether this is for example related to the much higher proliferative activity in the small bowel remains to be investigated.

P89

Systematic Audit of Upper GI Cancer Biopsies: Are We Missing Opportunities for Earlier Diagnosis?

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During the last year in Leeds, cancer was diagnosed in 193 out of 3737 gastric and oesophageal biopsy specimens (5.2%). These were taken by 70 endoscopists working in 4 endoscopy suites. We propose a routine audit should be carried out for quality assurance to avoid missed/delayed diagnosis of malignancy.
We piloted an audit of previous endoscopy in patients with a biopsy diagnosis of cancer as a quality assurance tool for the endoscopy service.
A monthly list of upper GI cancer was checked against the endoscopy database to identify patients who had had a previous endoscopy in Leeds in last 2 years. We reviewed endoscopy and biopsy reports of previous examinations, and histology slides of negative biopsies.
The results cover a 6 month period, March-Aug 2009. There were 95 patients with a biopsy diagnosis of gastric/oesophageal cancer; 68 had not had an endoscopy in the previous 2 years. Of the 27 who had an endoscopy within the last 2 years 12 had a previous biopsy diagnosis of cancer and 15 had a previous negative endoscopy.

	Number	Comment
New diagnosis of cancer with a non diagnostic endoscopy in the last 2 years	15	
Previous biopsy suspicious	0	
Previous biopsy dysplasia	4	Dysplasia in Barrett's (2 high grade, 2 low grade)
Previous biopsy not suspicious-focal lesion at endoscopy	2	5 & 8 pieces, 3 levels, reviewed-no malignancy
Previous biopsy not suspicious -no focal lesion at endoscopy	1	Barrett's no dysplasia
No biopsy - previous endoscopy suspicious	4	Had early repeat endoscopy
No biopsy - previous endoscopy not suspicous	3 no lesion, 1 failed procedure	

In conclusion 15/83 (18%) new cancer patients had non-diagnostic endoscopy in preceeding 2 years. Review of endoscopy/histology can identify avoidable factors in delaying diagnosis and provides quality assurance of the service.

P90

Invasive behaviour of the OE33 oesophageal adenocarcinoma cell line is dependent on co-operation with fibroblasts.

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BACKGROUND: Cell-cell and -matrix interactions play a critical role in influencing tumour cell behaviour, but conventional culture systems fail to capture these aspects of the tumour microenvironment. To investigate the pathogenesis of oesophageal adenocarcinoma we have developed a tissue-engineered model of the oesophagus which includes epithelial and stromal cells and a microanatomically realistic extracellular scaffold. In this study we examined the impact of fibroblasts on tumour cell behaviour. MATERIALS & METHODS: OE33 oesophageal adenocarcinoma cells were seeded on the superficial surface of sterilsed, decellularised porcine esophageal mucosa, in the presence or absence of fibroblasts on the deep surface. All experiments were repeated with two different media: RPMI and a 3-stage media with increasing calcium that we have previously used in culturing normal oesophageal squamous epithelium. Tissue cultures were maintained at air-liquid interface for 14 days. The resultant tissues were fixed and examined histologically. RESULTS: In all conditions the tumour cells proliferated and formed epithelial and glandular structures, histologically in keeping with a moderately differentiated adenocarcinoma. Non-invasive extension of the tumour into pre-existing ductal and glandular structures was frequently observed. Experiments incorporating fibroblasts showed extensive destructive invasion of the tumour into the extracellular matrix. Where fibroblasts were absent there was damage to the collagen matrix beneath the basement membrane, but tumour cells did not invade and instead remained on the tissue surface above the damaged matrix. DISCUSSION & CONCLUSIONS: These results indicate an essential role for fibroblasts in indirectly promoting invasive behaviour in tumour cells, and demonstrate the power of this experimental system to model aspects of tumour behaviour that are not captured in conventional culture systems.

P91

CTEN Modulates Integrin-Linked Kinase (ILK) /Focal Adhesion Kinase (FAK) Signalling and Nuclear Localisation is Associated with Liver Metastasis in Colorectal Cancer

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C-terminal tensin-like (Cten) molecule acts as an oncogene in colorectal cancer (CRC) and is associated with alterations in cell motility. We initially used small interfering RNAs to knock down Cten in the CRC cell line SW620. This resulted in inhibition of cell migration (P<0.001) and invasion (P<0.001) and up-regulation of E-cadherin. We hypothesised that the mechanistic link between Cten and E-cadherin may lie in Integrin-linked kinase (ILK) and focal adhesion kinase (FAK) as these are known to reduce E-cadherin levels. Cten forced expression in HT116 cells and knocked-down in SW620 cells resulted in up-regulation and down-regulation of ILK, FAK and activated phosphoorylated-FAK (p-FAK) respectively. In order to confirm the interdependence of FAK and p-FAK with Cten, we examined a series of 40 cases of paired primary CRC and hepatic metastases using immunohistochemistry. This showed a significant positive correlation between Cten and both FAK and p-FAK (P<0.001 and P<0.002 respectively). Both primary and metastatic tumours showed similar levels of Cten cytoplasmic staining. However, nuclear Cten expression was significantly greater in the metastatic deposits than the primary tumours (P=0.002). Finally, Cten expression was evaluated in 462 cases of CRC using immunohistochemistry and tissue microarray. Cytoplasmic Cten expression was seen in 92% of the tumours and was significantly associated with advance Dukes stage (P=0.001), lymph node metastasis (P<0.001), extra-mural vascular invasion (P=0.001) and distant metastases (P=0.008). Kaplan-Meier curve demonstrated that patients with high Cten expression had significantly shorter disease free survival (P<0.001) than those with low Cten expression. We conclude that Cten expression in CRC could have a role in prognostic assessment and therapeutic planning and its role in cancer metastasis may be mediated through modulation of the ILK and FAK signalling pathways.

P92

CD133 Expression in Colorectal Cancer and its Prognostic Significance

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Background: CD133 is a glycosylated cell surface molecule which has been controversially, reported as a CSC marker in colorectal cancer. In this study, we sought to study the expression and the prognostic value of CD133 in a large series of CRC. Methods: The prognostic value of CD133 expression was studied in a large series of colorectal carcinoma using tissue microarray and immunohistochemistry. Association between CD133 expression and clinicopathological variables and patient outcome were investigated. Results: An association was found between CD133 expression and patient's survival. It was found that CD133 negative expression associated with better survival, although it is not statistically significant but there is a trend. Moreover, multivariate analysis showed that CD133 was not an independent prognostic marker. Conclusion: In this series, CD133 was found not to be a good prognostic marker in colorectal cancer

P93

A Case of Epithelial Dysplasia Arising in the Abdominal Wall Following Loop Colostomy Formation

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We report a case of a 77 year old female who presented in 2009 with a cystic mass in relation to a colostomy scar dating back to emergency surgery for an obstructing diverticular stricture 20 years earlier. An ultrasound scan showed a suspected recurrent incisional hernia. A previous incisional hernia at the same site had been repaired with mesh 10 years earlier at which time a small mucinous cystic lesion was found. Microscopy of this showed fragments of benign mucinous epithelium thought to represent residual colonic epithelium from the colostomy.

At subsequent operation in 2009 a further mucinous cystic lesion was found in association with the residual mesh alongside a recurrent incisional hernia. Microscopy showed fibrous tissue partly lined by a columnar epithelium showing low grade dysplasia with no evidence of invasion. Immunohistochemical staining was positive for CK20, CEA and CDX-2 indicating large bowel origin.

The patient experienced a self-limiting post-operative ileus confirmed on CT scan but soon recovered and was asymptomatic at three months' follow up.

To the best of our knowledge, low grade dysplasia arising in a previously benign residual colonic epithelium from a previous colostomy has not been described before in the literature. Hernia sacs containing copious mucin and atypical epithelial cells within is a common finding in pseudomyxoma peritonei however in this case the cysts were discrete and localised with no clinical or radiological evidence of pseudomyxomatous disease.

P94

The Expression of RNA Binding Proteins in Colorectal Cancer

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The heterogeneous nuclear ribonucleoproteins (hnRNPs) are a group of RNA binding proteins with a range of key cellular functions which are dysregulated in tumourigenesis including regulation of translation and RNA processing. The purpose of this study was to define the hnRNP expression profile in colorectal cancer and establish the significance of hnRNP expression. A tissue microarray containing 515 primary colorectal cancers, 224 lymph node metastasis of colorectal cancer and 50 normal colon samples was immunostained for 6 hnRNPs. hnRNPI, hnRNPK and hnRNPL displaying the most frequent strong immunoreactivity in primary colorectal tumour samples. hnRNPA1 ($p<0.001$) and hnRNPU ($p=0.003$) showed significant alterations in nuclear expression in tumours compared with normal while hnRNPA1 ($p=0.001$), hnRNPI ($p<0.001$) and hnRNPK ($p<0.001$) all showed significant increases in cytoplasmic immunoreactivity in tumour cells. There were significant differences in cytoplasmic immunoreactivity between the primary tumour and the corresponding lymph node metastasis for hnRPA1 ($p=0.001$), hnRNPI ($p<0.001$) and hnRNPK ($p=0.001$). There was a significant relationship between strong nuclear hnRNPH expression and survival (chi-squared=14.97, $p<0.001$). This study has defined the expression profile of hnRNPs in colorectal cancer and shown that there are significant alterations in individual hnRNP expression in this type of tumour.

P95

Colorectal Cancer Biomarker Identification: A Proteomics Approach

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Colorectal cancer (CRC) is the third most common cancer in the UK and the second most common cause of cancer death, with a 5 year survival rate of 40-45%. Early diagnosis is essential for increased survival while improving response and outcome to chemotherapy may provide significant benefits to patients by reducing toxicity. Biomarkers have considerable potential to impact current understanding and outcome by providing prognostic or predictive information, act as screening tools or therapeutic targets. The purpose of this study was to profile protein expression in colorectal cancer. Colorectal tumour samples (n=29, all Dukes B) and normal colorectal mucosa were dissected within 30 minutes of resection and samples immediately frozen in liquid nitrogen and stored at -80oC. No patient had received any form of neo-adjuvant therapy. Proteins were separated using 2D gel electrophoresis. Gels were analysed using Progenesis Same Spots software for identification of differential protein expression between tumour and non-tumour tissues. Protein spots of interest were identified following in-gel tryptic digestion and analysis by liquid chromatography-tandem mass spectrometry. Of the 220 significantly differentially expressed proteins identified, 111 were up-regulated in tumour tissue. Initial analysis indicates nucleophosmin (2.1fold change, $p<0.001$), 14-3-3 protein (1.2 fold, $p=0.004$), translationally controlled tumour protein (1.6 fold, $p<0.001$) among others, are all up-regulated in colorectal tumour tissue compared to non-tumour tissue. Nucleophosmin, 14-3-3 protein and translationally controlled tumour protein are amongst a number of potential biomarkers of colorectal cancer identified using a 2D gel electrophoresis and mass spectrometry proteomic approach.

P96

Immunohistochemical Analysis of Colorectal Cancer with Gastric Phenotype: Claudin-18 is Associated with Poor Prognosis

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Claudin-18 plays a key role in constructing tight junctions, and altered claudin-18 expression has been documented in various human malignancies; however, little is known about the biological significance of claudin-18 in colorectal cancer (CRC). The aim of this study is to investigate the significance of claudin-18 expression in CRC and its association with clinicopathological factors. We performed clinicopathological analysis of claudin-18 expression in a total of 569 CRCs by immunohistochemistry. Moreover, we investigated the association between claudin-18 and various markers determining gastric/intestinal phenotype (MUC5AC, MUC6, MUC2, CD10 and CDX2). Claudin-18 expression was detected in 21 of the 569 CRCs (4%) and was seen exclusively on the cell membrane. Corresponding non-neoplastic colorectal mucosa and colorectal adenomas did not express claudin-18. Expression of claudin-18 was not correlated with T grade, N grade, staging, or histological type. Positive rates of various markers determining gastric/intestinal phenotype in 569 cases are as follows: 86 (15%) cases for MUC5AC, 11 (2%) cases for MUC6, 370 (65%) cases for MUC2, 200 (35%) cases for CD10 and 448 (79%) cases for CDX2. Positive expression of claudin-18 showed a significant correlation with positive expression of MUC5AC and negative expression of CDX2. The prognosis of patients with positive claudin-18 or MUC5AC expression was significantly poorer than in negative cases. In contrast, the prognosis of patients with negative CDX2 expression was significantly poorer than in positive cases. Multivariate analysis revealed that T grade, M grade and claudin-18 expression were independent predictors of survival in patients with CRC. In summary, we revealed that claudin-18 expression correlates with poor survival in patients with CRC and is associated with the gastric phenotype. Claudin-18 may be a useful marker to predict CRC and its prognosis.

P97

Diagnostic Accuracy of Endoscopic Ultrasound Guided Fine Needle Aspiration of Pancreatic Lesions

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*Purpose of Study*Endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) of the pancreas allows the evaluation of pancreatic neoplasms without the need for exploratory surgery. We review our recent experience of EUS-FNA and report the diagnostic accuracy.
*Methods*Data was collected from cytology reports for all patients who underwent EUS-FNA of pancreatic lesions between 1st January 2008 and 31st November 2009. Subsequent histology reports were reviewed where available to assess the diagnostic accuracy of the preceding cytology. We compared our results with those reported in the literature.
*Summary of Results*51 patients underwent EUS-FNA. 82% of samples were adequate for cytological assessment. 65% of these were given a benign cytological diagnosis and 35% were given a neoplastic diagnosis. Subsequent histology was available for review in 32% of cases. The sensitivity, specificity and accuracy of EUS-FNA for a neoplastic diagnosis were 78%, 100% and 85%, respectively. Studies from the literature report ranges of 64-94% for sensitivity, 88-100% for specificity and 74-94% for accuracy.
*Conclusions*EUS-FNA is an accurate method for the evaluation of pancreatic neoplasms. Our centre has limited experience of EUS-FNA and we have identified a need for improvement in the diagnostic accuracy of the technique. Our recommendations to improve the sensitivity and diagnostic accuracy include; cytologists to attend the hepato-biliary multi-disciplinary team meetings; cytology technical support available in the radiology suite for specimen preparation and the completion of a pro-forma for all pancreatic EUS-FNA specimens to maximise the clinical and radiological information available for correlation.

P98

Gastric Lymphangioma

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Lymphangiomas are benign vascular tumour that usually arise in the neck, head and axilla of infants. Gastrointestinal lymphangioma is a solitary, localized benign tumour composed of multiple dilated lymphatic channels of various sizes lined by endothelial cells. The pathogenesis has been presumed to be sequestered lymphatic tissue that is separated from the normal lymphatic system. These are extremely rare with an incidence of 1 per 50,000 subjects examined by GI radiography. There have been very few cases of gastric lymphangiomas reported in the literature. Most of the GI lymphangiomas occur in the intestine and they are rarely found in the stomach. The location and rare occurence makes this tumour a diagnostic challenge. When present near the lesser curvature these can be confused with pancreatic pseudocysts, gastrointestinal stromal tumours and other pancreatic related cystic tumour radiologically. We present a rare case of gastric lymphangioma which includes its clinical, radiological, intraoperative and histological findings.

P99

Audit of Changes in Diagnostic Practice of Hyperplastic Type Lesions in the Colon

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Aims Hyperplastic polyps (HPs) are benign but several subtypes and mimics of HP have been described, including sessile serrated adenoma (SSA). Molecular studies suggest that SSAs may have a malignant potential but the distinction from HP is problematic and several papers describe high inter- and intra-observer variability. The overall aim of this study was to determine the overall and site specific (right versus left colon) incidence of SSAs in a primary referral centre and secondarily to determine the robustness of the histological diagnosis of SSA versus HP. Methods All 299 cases of 'HP' from 2001-2 (prior to 2003 where SSA was first described in detail) were examined by a consultant pathologists and a medical student. All cases considered possible SSA were then reviewed by a second pathologist. Both pathologists were blinded to the site. Finally, all biopsies from the right colon, regardless of initial diagnosis, were reviewed by the 2 pathologists. Results 271 of 299 cases were included (others were discarded due to other pathology). Initially 27 biopsies were identified as possible SSAs but when reviewed by a second pathologist both agreed on 16 biopsies (overall incidence 6%) which included a second review of all biopsies from the right colon. SSAs accounted for 37% of all biopsies in the right colon (7 of 19) and 3.5 % of all biopsies in the left colon (9 of 252). Conclusions SSAs are more common in hyperplastic lesions of the right colon (37% vs 3.5%). The differential diagnosis between HP and SSA is difficult and the most useful criteria were architectural features. Until diagnostic criteria are in common use and the biological potential is fully known, SSA remains a problematic entity.

P100

Monoclonality and Immunophenotype of Intraepithelial Lymphocytes in Lymphocytic Gastritis

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Lymphocytic gastritis (LG) is an uncommon condition associated with autoimmune and infective diseases [e.g. H pylori, coeliac disease (CD), Crohn's disease) characterized by an increased number of intraepithelial lymphocytes (IELs) in the foveolar epithelium, primarily CD8+ T-cells. Its pathogenesis is poorly understood and the presence of monoclonal T-cell populations and the potential risk of T-cell lymphoma (similar to Enteropathy-associated T-cell lymphoma) have not yet been investigated.
Methods: The study included 27 cases of LG. Clinical information regarding H pylori (Hp) infection and CD was recorded. Immunohistochemistry for Hp, CD3, CD4 and CD8 and PCR amplification of the T-cell receptor (TCR) gamma gene was performed in each case.
Results: 8/27 cases were positive for Hp and 2/27 had histologically confirmed CD. In all cases the IELs showed a $CD3^+/CD8^+$ immunophenotype. In 19/27 cases DNA quality allowed PCR analysis and in 4/19 cases a monoclonal expansion of T-cells was seen. Two cases were associated with Hp and the remaining cases had no evidence of Hp infection or CD. In one case there was clinical / endoscopic improvement after Hp eradication and in 1/4 LG persisted despite treatment. None of the patients have developed lymphoma (median follow up 21 months).
Conclusion: Hp infection may trigger LG and induce infiltration of T lymphocytes in the gastric mucosa. In a significant proportion of cases (14% in our series) a T-cell clonal expansion may develop and in these cases repeat biopsy and close follow up should be mandatory. Some patients with monoclonal T-cell expansion are not H pylori-related and other host-related factors may play a role. It is important to include immunohistochemical analysis and TCR gene rearrangement tests in the diagnosis of lymphocytic gastritis

P101

Gain of Chromosome 13 is a Cause of *CDK8* Overexpression Associated with Colorectal Adenoma to Carcinoma Progression

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Introduction: Colorectal adenomas are common precursors of colorectal cancer (CRC). About only 5% of adenomas progress to cancer and this progression is mostly associated with overall onset/increase of chromosomal instability. Gain of 13q is often implicated in this progression of adenoma to carcinoma (1). Until recently however no genes were identified to be the drivers of this amplicon. Recently, Firestein and collaborators (2) showed that cyclin 8 (*CDK8*), at 13q12.13, functions as an oncogene in CRC.
The aim of this study was to evaluate *CDK8* gene dosage effects in colorectal adenoma to carcinoma progression.
Material and methods: Sixty seven colorectal tumours (34 adenomas and 28 carcinomas) were analysed by array CGH (5k BAC platform, including contig coverage of 13q) and by expression microarray (30k Compugen library). Integration of DNA copy number dosage and gene expression was performed using the Ace-it tool (3).
Results: In the tumours analysed we observed 13q copy number gain in 9% and 46% of adenomas and carcinomas, respectively. Integrating copy number and mRNA expression with the differential upregulation of genes between carcinomas and adenomas, provided us with a list of 43 genes. Within this list, CDK8 ranked 7th in significancy ($p=0.003$). Upregulation of *CDK8* was confirmed by real-time RT-PCR.
Conclusions: Copy number gain of 13q has a gene dosage effect on *CDK8* mRNA expression, indicating a role of this gene in colorectal adenoma to carcinoma progression.
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(2) Firestein R et al. CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity. Nature 2008 Sep 25;455(7212):547-51.
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P102

Audit of Colorectal Cancer Pathology Reporting in Kent & Medway Cancer Network: Compliance with National Guidelines

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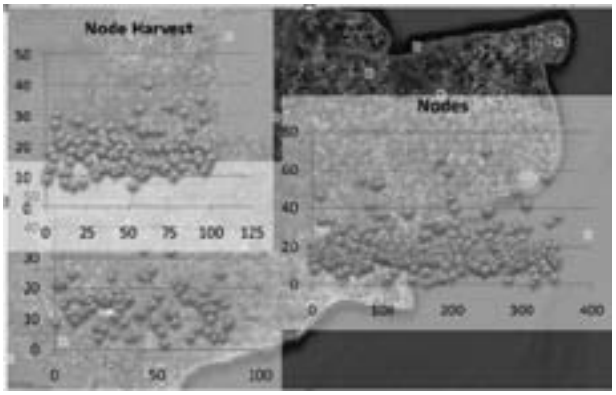
Introduction: There is evidence in the literature of great variability in reporting of colorectal cancer pathology, which clearly can impact therapeutic decisions and prognosis prediction. We audited the compliance of pathology reporting, across Kent and Medway cancer network, with national guidelines. Patients and methods: 519 Patients with Primary colorectal adenocarcinomas treated with radical cancer resections in Kent and Medway cancer network from April 2008-April 2009 were included in the study. Data collection: Minimum data set paper forms, pathology reports and Pathosys database were used, with data entered into Bento 3 database and then exported to Excel Spreadsheet for analysis. Data collected: Size, stage, type, grade and appearance of the tumour, depth of invasion & presence of tumour perforation. Total number of lymph nodes excised and number involved. Tumour involvement at surgical margins, including circumferential margins and in rectal cancers involvement of distal margins and quality of mesorectal resection. Presence of extramural venous invasion. Whether Neoadjuvant therapy was used or not prior to surgery. Results: 12 nodes or more were harvested in 75-85% of patients. A median of 16-18 nodes were harvested per case across the network. Positive nodes were found in 41% - 50% of specimens.

All reports mentioned whether margins were clear or involved. Reporting on circumferential margins was variable. Some variations also occurred in reporting Quirke grade in rectal specimens, which was not reported in all rectal cases.

Colonic and rectal serosal involvement was reported in 30-35% and 14-17% of specimens respectively across the network. Extramural venous invasion was reported in all patients. It was positive in 37-49% 0f cases. Conclusions: Colorectal pathology reporting fulfilled the minimum data set across the network.



Variation in the Reporting Format

Dataset	EKHT	MTW	MEDWAY	DARTFORD
Size	Same	Same		
Stage	Same	Same		
Type & Grade	Same	Same		
Depth of invasion	Generally reported for T3 only			
Lymph nodes	Same	Same		
Extramural Venous Invasion	Same	Same		
Surgical margins	Not reported in details by many pathologists. Report concentrating on involved or near margins			
Circumferential margin involvement	Different ways of reporting, circumferential, radial, deep, anterior, posterior, lateral			
Quirke grade	Not reported universally by all pathologists			

P103

A Case of Muir-Torre Syndrome

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We report a case of Muir-Torre syndrome (MTS), an autosomal dominant disorder with variable phenotypic expression characterised by the association of sebaceous gland neoplasms and visceral malignancy, usually colon cancer. It is a rare condition with just over 205 cases identified in a recent review. The patient was a male aged 56, who attended for routine colonoscopy in 2009, following abdominoperineal resection for adenocarcinoma of the sigmoid colon in 1985. This showed an annular tumour in the transverse colon. Subsequent right hemicolectomy showed a Dukes' C2 adenocarcinoma. Because of the history of metachronous carcinomas of bowel and his relative youth at the time of the first carcinoma, his previous medical history and family history were investigated. A sebaceous adenoma of the eyelid and a basal cell carcinoma with sebaceous differentiation of the nipple had been resected in 2001 and 2008 respectively. There was a strong family history of bowel cancer, the patient's mother, maternal uncle and two maternal cousins being affected. The most common skin tumours associated with MTS are sebaceous carcinomas, sebaceous adenomas and basal cell carcinomas with sebaceous differentiation. The most common visceral malignancy found in MTS patients is adenocarcinoma of the large bowel. The diagnosis of sebaceous gland neoplasms precedes visceral cancer diagnosis in 22% of cases, occurs concurrently in 6% and after visceral malignancy in 56%. Cutaneous findings can precede the diagnosis of internal disease by up to 25 years or follow it by up to 37 years. The genetic basis for MTS is defects of mismatch repair proteins (MMR). The importance of this condition is that diagnosis is often delayed and in some cases missed. The onus for prompt diagnosis lies mainly with pathologists and dermatologists.

P104

Villous Adenoma of the Bladder: Vogelstein Revisited?

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Villous adenoma of the bladder is a rare lesion that morphologically resembles villous adenoma of the colon. We present a case of a 71-year-old lady with a history of recurrent urinary tract infections. Computed tomography scan revealed a polypoid bladder mass and on cystoscopic examination a 4 x 5 cm mucus-secreting exophytic tumour was identified at the dome. Transurethral biopsies showed a papillary tumour composed of dysplastic enteric-type glandular epithelium consistent with a villous adenoma. The patient underwent further deep resection of the residual tumour and on this occasion foci of invasive mucinous adenocarcinoma were identified in association with villous adenoma. The patient went on to have a radical cysto-urethrectomy which confirmed the presence of a large villous adenoma with focal invasive adenocarcinoma of stage pT3. Although, in its pure form, villous adenoma of the bladder has an excellent prognosis with complete resection being curative, a significant proportion of cases have been reported to show co-existent invasive malignancy, with a risk of nodal spread and distant metastases. This case highlights the importance of complete resection of villous adenomas, as invasive carcinoma may not be apparent on initial biopsies. Where invasive carcinoma is identified, clinical and radiological correlation is essential to exclude direct invasion or metastasis from other primary sites including colon or female genital tract, which may show similar morphological and immunohistochemical features. Finally, the presence of adenocarcinoma arising within a villous adenoma of the bladder suggests an adenoma-carcinoma sequence of tumourigenesis similar to that identified in the colon. However, whilst the latter has been extensively studied at a molecular biology level, relatively little is known about the sequence of events in the bladder. Further research in this field is required.

P105

Epithelioid Haemangioma of the Penis

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A 50-year-old man presented with a 6-month history of a small painful nodule on the dorsum of his penis. Examination by ultrasound showed a well-circumscribed 8mm nodule within the subcutaneous penile tissue, superficial to corpus cavernosum, with evidence of vascular flow. The nodule was resected under general anaesthetic and histopathological examination revealed a lesion composed of sheets of plump epithelioid cells with evidence of vessel formation, particularly at the periphery of the lesion. There was a florid infiltrate of lymphocytes and eosinophils, but no significant cytological atypia and only a few mitoses. Immunohistochemistry showed the epithelioid cells were positive for CD31 and CD34 and negative for S100 and desmin. A diagnosis of epithelioid haemangioma was made. At 3 month follow-up there was complete resolution of symptoms with no evidence of local recurrence. Epithelioid haemangioma is an uncommon vascular lesion of uncertain aetiology and pathogenesis, most commonly arising on the head or distal extremities. Rare cases involving the penis have been reported. The lesion behaves in a benign manner and is best treated by local excision with regular follow-up to monitor for local recurrence. Clinically, it is important to distinguish this condition from Peyronie's disease, which is not normally biopsied or excised. Histologically, exuberant examples of epithelioid haemangioma can potentially be misdiagnosed as malignant epithelioid vascular tumours such as epithelioid haemangioendothelioma or epithelioid angiosarcoma.

P106

Sequence Variation in the SDHD Gene in Renal Oncocytoma

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Succinate dehydrogenase (SDH) is a nuclear encoded mitochondrial protein involved in both the Kreb's cycle and the electron transport chain. Heterozygous germline mutations in the genes encoding SDH subunits B, C and D are known to cause hereditary paragangliomas and phaeochromocytomas. Renal cell carcinomas (RCC) have also been described in around 4% of patients with germline SDHB mutations. Recently SDHB mutation has been described in oncocytoma and oncocytoma like renal tumours. DNA was extracted from 28 formalin fixed paraffin embedded (FFPE) sporadic renal oncocytomas (RO) and 4 chromophobe renal cell carcinomas (ChRCC) samples and analysed. Adjacent normal renal cortex DNA was extracted to allow demonstration of LOH. The use of these tissues for our research was approved by the Ethical Committee of the Regional Tissue Bank. Validated and SNP checked primer sets were available in the laboratory for the SDHB and SDHD genes that include all coding regions and intron-exon boundaries A panel of microsatellite markers close to the SDHB and SDHD loci on chromosomes 1 and 11 respectively were used to examine RO tissue for evidence of LOH. No significant mutations were identified in the SDHB gene. However, two samples from the 21 RO cases that were successfully sequenced for SDHD exon 2 were found to be heterozygous for the SDHD c.149A>G / p.His50Arg variant (9.5% RO, 0 from ChRCC samples). This variant was also present in normal tissue from each patient, representing a germline change. This variant has a very low frequency in the general population. There was no evidence of LOH at the any of the chromosome 11 markers flanking SDHD. LOH for chromosome 1 was found in a proportion of RO but this is a well described event. In conclusion we have identified germline sequence variation in the SDHD gene in a proportion of renal oncocytoma patients.

P107

Stem Cell Niches in the Bladder? The Clonal Organization of Human Urothelium.

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Purpose of the study: The urothelium is composed of 5-6 layers of cells; the basal cells, an intermediate compartment and a superficial layer of "umbrella" cells. Proliferation is usually restricted to basal layers, where the urothelial stem cells are thought to reside, but this has never been experimentally demonstrated. The clonal composition of normal urothelium is important in understanding how tumours begin to develop if we subscribe to the stem cell theory of carcinogenesis. Little is known about the stem cell population or clonal architecture of normal urothelium, therefore we wanted to visualize the clonal progeny of urothelial stem cells with the following methods. Methods: Histochemistry for mitochondrial enzyme cytochrome c oxidase (CCO) and succinate dehydrogenase was performed on multiple frozen sections of cystectomy specimens (n=9). CCO-active and -deficient areas were laser-capture microdissected. The entire mitochondrial genome (mtDNA) was amplified using a nested PCR protocol and subsequently sequenced for mtDNA mutations. Summary of results: CCO-deficient areas could be observed in normal urothelium of all cystectomies. The 2-dimensional length of these negative patches varied from 2-3 cells (about 2nm) up to areas of about 3mm. Each cell within a CCO-deficient region contained an identical mtDNA mutation, indicating a common stem cell of origin. Conclusions: Our results demonstrate that normal human urothelium contains markedly varying clonal units maintained by stem cells. These stem cells are capable of generating patchy areas with all differentiation characteristics. It is probable that each clone shows the site of a stem cell niche.

P108

Caveolin-1; an Oncogene that Promotes Growth and Invasion of RCC Cells via Potentiation of AKT/mTOR and MAPKinase Signalling

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Immunohistochemical studies of Renal Cell Carcinoma (RCC) have shown that the over-expression of caveolin-1 (cav-1) correlates with increased tumour size, high tumour grade, presence of vascular invasion and shorter disease-free survival. However, the molecular mechanism(s) underlying the role of cav-1 in RCC pathophysiology remains unresolved. In a panel of clear cell RCC cell lines we have examined signalling mechanisms underlying the contributions of cav-1 to RCC cell growth and invasion.

The siRNA targeted down-regulation of cav-1 led to decreased cell growth in all RCC cell lines examined, with respective reductions of 30% and 50%, respectively in the primary, VHL (—ve), 786-O and A498 cell line, and a 75% reduction in growth in the metastatic VHL (+ve) caki-1 cell line ($p<0.001$). Using Matrigel™-coated chambers the inhibition of cav-1 decreased the invasive capacity of the RCC cell lines ($p<0.05$). Immuno-blotting revealed that silencing of cav-1 caused reduced expression of phosphorylated (activated) forms of AKT, S6K1 and ERK paralleled by lower expression levels of the down-stream mTOR effector molecules eIF4E and cyclin D1. The of cav-1 inhibition upon growth, invasion and altered cell signalling were demonstrably greatest in the caki-1 cell line.

This study shows for the first time that cav-1 is an important oncogene in RCC promoting tumourigenesis through increased cell proliferation and invasion and that cav-1 promotes the activity of AKT/mTOR and MAPKinase signalling pathways. We conclude that cav-1 represents a novel target for the treatment of advanced RCC disease and underlines its importance as a biomarker for RCC progression

P109

Morphology of Recurrent Dense Deposit Disease is Not Typical of Native Disease: A Report of Two Cases

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Dense deposit disease (DDD) commonly recurs in renal allografts; this may result in graft failure and correct diagnosis has important implications for immediate treatment and management of subsequent grafts. We present two females with primary renal failure due to DDD that illustrate the varied morphology; one was treated by rituximab.

Case 1 had a renal allograft at 7 yrs after developing renal failure at 11 yrs age. At 14 months an acute increase in creatinine was investigated by renal biopsy. Histology showed an acute diffuse endocapillary glomerulonephritis with no GBM abnormality on silver stain and no interstitial inflammation. C3 was present on capillary loops; electron microscopy (EM) showed typical linear dense deposit. Treatment with plasmapheresis resulted in poor but stable renal function for the next 6 months.

Case 2 developed renal failure at 20 yrs age and had a renal allograft at 23 yrs. A biopsy at 3 weeks for secondary dysfunction showed glomerular intracapillary thrombus only. Biopsy at 7 weeks showed an acute focal segmental proliferative GN without significant scaring or tubulo-interstital changes. The GBMs were normal on silver stain; however on EM linear dense deposits were seen. Treatment with plasmapheresis and rituximab resulted in clinical recovery; a protocol biopsy carried out at 28 weeks showed focal segmental sclerosis with no active lesions and resolution of the dense deposit at EM. At 38 weeks relapse resulted in further decline in renal function.

Recurrent DDD rarely presents with a classical MPGN histological pattern; intracapillary thrombi and a focal segmental proliferative GN appear to be novel findings. This variety of morphologies emphasises the importance of electron microscopy. Initial success, including histological resolution, with rituximab is encouraging; further assessment of this treatment can only be made on a multicentre basis.

P110

Tubular Amyloid Casts in Myeloma

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Myeloma may result in a variety of renal lesions. Cast nephropathy is the result of precipitation of monoclonal light chains in the renal tubules; occasionally these may form crystalline structures. The deposition of amyloid, also due to light chain organisation, usually occurs in the glomeruli or interstitial space and not in lumen of the tubules. To your knowledge there are only three previous reports of tubular amyloid casts. We here report two cases in association with multiple myeloma.

Case 1: 71 yr. old male with 3.9 gm proteinuria and rising creatinine to 268 was investigated with a renal biopsy. This showed soft eosinophilic casts in tubules at the cortico-medullary junction with a prominent giant cell reaction. These stained strongly with congo red and showed strong red-green birefringence. Subsequent investigation confirmed myeloma with Bence Jones protein in the urine.

Case 2: 83 yr. old male with known myeloma, 5gm protinuria and rising creatinine underwent a renal biopsy. Findings were similar to those in Case 1 except that the amyloid like characteristics were restricted to the periphery of the casts.

Electron microscopy confirmed the fibrillary nature of the casts in both cases and in addition demonstrated probable amyloid deposition in glomeruli in case 2, not apparent on light microscopy. The diagnosis of a cast nephropathy in these cases was not in doubt; however in many cases the sparse distribution of the casts may make diagnosis challenging. An awareness of the possibility of tubular amyloid casts as a phenomenon may help the pathologist reach a diagnosis.

P111

EIF4E and Caveolin-1 Co-operate to Drive the Aggressive Features of Clinically Confined RCC

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EIF4E is an important translational regulator that acts down-stream of the AKT/mTOR pathway. It is associated with chemo-resistance and inhibition of p53-induced apoptosis. We have recently shown in a panel of RCC cell lines that caveolin-1 (cav-1) is an important oncogene in RCC that serves to promote the over-expression of eIF4E. However, no information exists regarding the clinical significance of EIF4E or is potential association with cave-1 in RCC.

Immunohistochemistry for eIF4E and cav-1 expression was performed on tissue microarrays (TMAs) constructed from 174 clinically confined RCC cases. Specimens were scored semi-quantitatively according to previously validated criteria and converted to a binary simple covariate (positive or negative) according to the most informative split on Kaplan-Meier using the log-rank statistical test. A significantly decreased mean free-survival was observed when eIF4E was co-expressed with cav-1 (2.6 vrs 6.0 yrs; p= 0.001) compared to when either EIF4E (4.3 vrs 6.2yrs; p=0.002) or cav-1 (4.7 vrs 6.2yrs; p=0.013) was expressed alone. On multivariate analysis, the composite co-variate of 'eIF4E/cav-1' was a significant influential indicator of poor disease free-survival with a hazard ratio of 3.4 (95% CIs: 1.6-7.1). Tumours that co-expressed eIF4E and cav-1 were more likely to be larger, of higher grade and show vascular invasion.

These results provide clinical evidence that eIF4E and cav-1 co-operate to drive disease progression in primary RCC tumours and that the cav-1/AKT/eIF4E axis may be an important molecular target for preventing metastasis and restoring chemo.-sensitivity.

P112

WT1, PTEN and phosphoPTEN Staining in an Ovarian TMA

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WT1 was first described as a tumour suppressor gene in Wilms tumour, classically WT1 is now used as a marker of ovarian serous adenocarcinoma and is thought to be largely negative in the other ovarian malignancies. PTEN is the second most common tumour suppressor gene in human cancer and acts as a negative regulator of the AKT pathway which controls cell growth and proliferation. We created an ovarian TMA containing a variety of different ovarian malignancies & stained it with both the WT1 C19 and WT1 6F-H2 antibodies, PTEN and pPTEN antibodies. Each core was scored (cytoplasmic and nuclear staining) from 0 – 3. There was a significant difference between WT1 C19 and WT1 6F-H2 staining (n to n staining p = 1.29862x10-15), with non specific staining seen in WT1 C19. Table 1 shows the overall mode of each antibody together with the serous carcinomas.

	WT1	6F-H2	PTEN		phosphoPTEN	
	Cytoplasmic Staining	Nuclear Staining	Cytoplasmic Staining	Nuclear Staining	Cytoplasmic Staining	Nuclear Staining
Overall Mode Total Tumours	0	0	0	0	1	0
Overall Mode Serous carcinoma	0	1	1	0	1	0

There is a significant difference between WT1 nuclear staining and PTEN nuclear staining (p= 0.00065),& WT1 nuclear & PTEN cytoplasmic staining (p=7.64921x10-18). Overall there appears to be similar staining of the active PTEN and the inactive pPTEN but there is a significant difference seen between cytoplasmic staining (p= 0.00020) it would appear that WT1 6F-H2 does stain positively in the majority of ovarian serous carcinomas 73% of cases stained 1-3 nuclear positivity, however the majority of these cases displayed weak staining only. While 97% of cases showed no cytoplasmic WT1 staining. 64% of cases showed absent PTEN nuclear positivity 58% showed weak cytoplasmic PTEN positivity and 36% no staining. 94% of cases showed absent nuclear pPTEN staining 55% and 28% showed grade 1 and 2 cytoplasmic staining. In light of the significant differences between WT1 and PTEN there is evidence to suggest that they may play different roles and may even interact with each other, and further investigation is required to to examine this.

P113

"Cadherin Switch" in Epithelial Ovarian Carcinoma

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Epithelial ovarian carcinoma (EOC) often has a poor prognosis due to late presentation of the disease at first diagnosis and development of chemoresistance. New targeted therapy is required if the survival in these cases is to improve. There is a growing body of evidence supporting the promising role of adhesion molecules in cancer targeted therapy considering their association with tumour progression. The profile of E,P and N cadherins in EOC and its association with survival remain poorly understood. Down-regulation of E-cadherin accompanied by up-regulation of either P orN-cadherin in advanced stages of cancer has been described as the "Cadherin Switch". We hypothesised that the behaviour of tumour cells is regulated by the "Cadherin Switch" in the scale of intensity of expression of the 3 cadherins. In order to identify the stages of the "Cadherin Switch" in EOC, we studied the immunoexpression of E, P and N-cadherins in a cohort of 311 cases of EOC. High expression of P-cadherin was associated with poor patient survival and was significantly higher in stage 2 in comparison to stage 1 and stage 3 disease (p=0.002). In contrast, loss of E-cadherin was observed in stage 3 EOC in comparison to other stages (p=0.046). Most interestingly, N-cadherin was a prognostic factor predicting survival independent of tumour stage (HR 0.558, 95% CI 0.325-0.960, p=0.035). Our results indicate the "Cadherin Switch" alters through progression of the EOC. Further, N-cadherin has an important role in determining the biological behaviour of ovarian cancer at an early stage before it is exposed to the influence of the new tumour microenvironment and being part of the Epithelial Mesenchymal transformation process and could potentially be used as a target for therapy.

P114

Grading of Endometrial Cancer: Concordance Between Pre-Operative Biopsy and Hysterectomy Specimens

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Introduction: Definitive histological grading of endometrial cancers is best undertaken on thoroughly sampled hysterectomy specimens. As grading of endometrioid lesions is dependent on assessing the relative proportions of solid and glandular components, thorough sampling minimises the risk of mis-grading due to sampling error. The initial diagnosis of endometrial cancer is usually made by examining pipelle biopsy or curettage specimens. Lesions can be provisionally graded using these biopsies but, due to potential sampling error, this grade cannot be regarded as definitive. Nevertheless, this provisional grade can inform as to the appropriate surgical management of the patient. Patients with a pre-operative provisional diagnosis of a Grade 3 lesion are likely to be considered for more aggressive surgical management, namely lymph node dissection to achieve definitive surgical staging, in addition to TAH&BSO. The aim of this study was to determine the concordance between the provisional pre-operative biopsy grade of endometrial cancers with the definitive post-hysterectomy grade.
Methods: The study was retrospective. All cases of endometrial cancer treated at Aberdeen Royal Infirmary between Jan 2003 and Feb 2009 in which a pre-operative biopsy was followed by a hysterectomy were identified. Histopathology reports were accessed and the pre-operative biopsy grade was compared to the definitive hysterectomy tumour grade.
Results: Of 243 cases in the study 238 had both a pre-operative biopsy grade and a definitive hysterectomy grade documented. Of these cases 44% were pre-operatively Grade 1, 21% Grade 2 and 34% Grade 3. The overall concordance rate with subsequent hysterectomy was 80%. Pre-operative biopsies graded 1, 2 and 3 showed concordances of 76%, 68% and 93% respectively. The concordance rate for pre-operative biopsies either Grade 1 or Grade 2 was 92%.

P115

Audit of the Reporting of Invasive Cervical Carcinoma in Loop Excision Specimens

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Careful reporting of loop excision specimens that contain invasive cervical carcinoma is important for determining the management, prognosis and follow up of patients. In this audit, reports were identified retrospectively using a computer-based search of the pathology reporting database at Ninewells Hospital, Dundee to retrieve all loop excision specimens that contained invasive carcinoma over the period from January 2002 to January 2010, which spans the introduction of proforma reporting in 2006, and modification of the dataset in 2008. Of the 70 reports identified, 19 used the 2008 proforma, 12 the 2006 proforma and 39 no proforma. Reports were anonymised and their content compared with the 2008 dataset. Introduction of the proforma in 2006 led to improvement in the inclusion of parameters describing the invasive tumour (type, size measurement, presence of lymphovascular invasion (LVI)), and the involvement of resection margins. The 2008 modification led to a further improvement in report content, with all 19 reports containing tumour type, differentiation, LVI, assessment of all tumour dimensions and all resection margins. However, the proportion of summaries of these reports that contained each of the 5 required parameters ranged from 53 — 95%. Whilst this represents an improvement over reports that did not use a proforma (10 — 41%) and those based on the 2006 dataset (17 — 92%), there is clearly room for improvement in this component of the report. In conclusion, the introduction of proforma reporting of loop excision specimens containing invasive cervical carcinoma has led to the inclusion of clinically important parameters in almost all reports. However, report summaries are often incomplete.

P116

Classical Carcinoid Tumour Arising from an Intestinal Structure Within a Mature Cystic Teratoma

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We present a case of an insular type carcinoid tumour arising within a recapitulated segment of bowel within a mature cystic teratoma. Well differentiated structures are commonly identified within mature cystic teratomas (MCT) but the presence of well formed organoid structures is relatively rare. Several studies have reported the presence of well-differentiated intestinal structures within MCTs, several of which appeared to give rise to separate neoplasms. A single case reported the presence of an intestinal structure within a MCT with a concomitant carcinoid tumour: however, the precise origin of the carcinoid tumour remained uncertain. The case presented provides strong evidence for an "intestinal" origin of the carcinoid tumour through immunohistochemical studies and on morphological grounds. The carcinoid tumour cells expressed CK7 and 19, which were also strongly co-expressed by the glandular epithelium lining the intestinal structure. Similarly, synaptophysin was expressed by neuroendocrine cells present in the lining epithelium and was strongly expressed by the carcinoid tumour. The presence of infiltrating islands of the carcinoid tumour within the apparent muscularis propria further support its origin from the segment of "intestine". This is an unusual case that highlights the diversity of findings present within the spectrum of mature cystic teratomas and provides evidence that carcinoid tumours arising in this context may be of true intestinal origin.

P117

An Audit Assessing the Adequacy of Liver Biopsy Specimens Sent Over a One Year Period.

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Background: Liver biopsy is an important tool in the evaluation of patients with liver disease. Biopsies are commonly collected via ultrasound guidance or through a transjugular approach. It is recommended that an optimal liver biopsy should be longer than 20mm or have 11 or more complete portal tracts (CPTs). This audit aimed to assess the proportion of liver biopsies collected over a one year period which met this standard. Method: Data was collected from the reports of biopsies collected over one year; data included patient demographics; the method used to obtain the biopsy; the number of cores taken, and the size and number of CPTs per biopsy. Cases where the samples were obtained from surgery were excluded. Results: Data was collected from 53 reports. 85% of the biopsies had been collected through ultrasound guidance. The mean age of the patients was 59.7 years. The mean length of the samples was 16.2mm; biopsies collected through ultrasound guidance were on average 16.7mm, the corresponding figure from transjugular specimens was 12.4mm. The specimens collected with ultrasound guidance were more likely to be in excess of 20mm than those collected through the transjugular approach (47% and 20% respectively). The number of CPTs was only documented in 13% of all the cases, in these specimens the mean number of CPTs was 8.4. Only 47% of the biopsies met our standard. Conclusion: Our results showed that less than half our sample met the standard. The standard was more likely to be met on the criterion of sample size. The mean number of CPTs was infrequently documented and therefore we recommend that the number of CPTs be routinely documented in reports. Furthermore, our data suggest that an ultrasound guided approach may be more likely to yield an adequate biopsy sample. However, due to our small sample size further study is required in this area to assess the adequacy of liver biopsy samples on a wider scale.

P118

Is Oil Red-O and Digital Image Analysis the Gold Standard for Quantifying Steatosis in the Liver?

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Background & Aims: The NAFLD Activity Score (NAS) is a well validated score for determining the severity of NAFLD using an H&E slide. The degree of steatosis contributes up to 3 of the 8 points to NAS and hepatocyte ballooning (which may be confused with fat) a further 2 points. Therefore, it is important to correctly identify steatosis in a liver biopsy. Oil Red-O (ORO) stain is the gold standard for identifying fat in tissue.

Our aim was to compare two pathologists with digital image analysis (DIA) using both H&E and ORO to assess the degree of fat in mice with NAFLD.

Methods: We studied histological steatohepatitis exhibiting a spectrum of steatosis in C57BL/6J mice with high fat and/or high fructose diets. Slides were stained with H&E and ORO and examined by two pathologists. DIA was used to calculate the percentage of steatosis on H&E and ORO stained slides and size distribution of fat droplets. Triglyceride concentrations in the tissue were measured biochemically as a true reflection of tissue lipid content.

Results: ORO staining identified fat in the control and fructose groups where no fat was identified by pathologists on H&E slides. Most of this fat was microvesicular.

The two pathologists showed strong interobserver agreement (Pearson correlation R=0.991, p=<0.001). However, compared to ORO DIA, they overestimated the amount of fat for the high fat (71.6% vs 46.2%) and high fat/fructose (71.9% vs 47.0%) groups where was mainly macrovesicular fat (p=0.011 and p=0.003).

ORO DIA accurately reflected the liver triglyceride concentrations and is therefore an accurate reflection of liver steatosis (R=0.706, p=0.001).

Conclusions: ORO staining can identify fat in cases where it cannot be seen on H&E staining. Although NAS is clinically valid, in research situations ORO DIA is warranted as the most reliable way to accurately assess liver steatosis and also give additional information on fat droplet size

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Recognising Histological Regression in Colorectal Liver Metastases Treated with Oxaliplatin Based Chemotherapy: Inter-Observer and Inter-Tumour Variation

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Neoadjuvant chemotherapy is used to increase the resectability of colorectal liver metastasis (CRLM). Can histopathologists distinguish chemotherapy response from 'biological' necrosis in CRLM? Method: Patients with CRLM who were chemo-naive or previously treated with oxaliplatin-based chemotherapy were identified. Tumour regression/fibrosis was scored using Mandard criteria adapted for CRLM (1), and the % tumour necrosis estimated, by 2 histopathologists unaware of previous treatment. We compared inter-observer variation using kappa statistics, and variability of tumour regression among multifocal tumours. Results: Inter-observer variation kappa statistic (197 tumour blocks) was 0.87 for major/ partial/no response and 0.81 for necrosis scored 0-4. Evidence of tumour regression by patient is shown in the the table.

	No chemotherapy (20)	Neoadjuvant chemotherapy (20)
Median age in years (range); male:female	73.5 (55-80); 14:6	58.5 (44-75); 11:9
Number with >1 tumour	11	12
Median number of tumours/case (range)	2 (1-7)	2 (1-21)
Median tumour diameter (range)	21mm (3 - 150mm)	18mm (4 - 80mm)
No regression, any tumour (score 4-5)	10	8
Partial regression at least 1 tumour (score 3)	9	4
Major regression at least 1 tumour (score 1-2)	1	8
Of regression, number with unresponsive tumour (score 4-5) elsewhere	8	7
Average necrosis score, all tumours	1.75	1.5
Cases with necrosis score >50%	12	10

Conclusion: Major tumour regression with fibrosis (score 1-2) was seen in 8/20 chemotherapy patients and 1 untreated patient; 15/22 with regression also had non-regressed tumours. Some tumour necrosis was universal and independent of chemotherapy. Inter-observer agreement was excellent. Features suggestive of chemotherapy response also occurred in untreated patients, and regression was usually heterogeneous in patients with multiple tumours. Reference: 1) Rubbia-Brandt et al, Annals of Oncology 18: 299-304, 2007

P120

Nodular Lymphoid Hyperplasia Occurring Concurrently in the Pancreas and Liver

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A 64-year-old woman presenting with weight loss was found to have a suspicious mass in the tail of the pancreas and a smaller mass in the liver following investigations. Two fine needle aspirations showed features suggestive of chronic pancreatitis, but an elective distal pancreatectomy and a non-anatomic liver resection were performed to exclude a neoplastic process. The liver mass was a fairly well-circumscribed pale nodule measuring up to 5mm; the pancreatic tail mass was ill-defined, stellate-like and pale measuring up to 26mm. Microscopically, both lesions were composed of lymphoid follicles with polarised germinal centres containing tingible body macrophages and surrounded by well-defined mantle zones. These were also present in peri-pancreatic adipose tissue and lymph nodes. There was associated fibrosis, and a mild chronic inflammatory cell infiltrate in the pancreatic tail but no evidence of periductal inflammation. Immunohistochemistry demonstrated preserved B and T cell compartmentalisation with bcl-2-negative follicles throughout. In-situ hybridisation demonstrated a polyclonal light chain population. The overall appearances were of nodular lymphoid hyperplasia, a benign, localised lymphoid proliferation, which produces a tumour-like lesion. It has been described in various anatomic locations; however, to our best knowledge this is a first description of it occurring concurrently in the pancreas and liver. There are reported associations with immune-mediated disorders and immunodeficiency, but these were absent in this patient. It is important to be aware of this phenomenon and to exclude low grade lymphoma. We advocate an avoidance of the term"pseudolymphoma" to describe what is essentially a reactive process.

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Case Report: Congential Hepatic Fibrosis Presenting in the Sixth Decade

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Congenital Hepatic Fibrosis (CHF) is a rare autosomal recessive fibropolycystic disease with hepatic manifestations of periportal fibrosis and bile duct proliferation, presenting in younger life with cholangitis or portal hypertension. We describe here the case of a 60 year old lady who presented with jaundice and right upper quadrant pain after a ride on the 'Big Dipper' at Blackpool pleasure beach. This was her first episode and her past medical history was unremarkable. Serum biochemistry showed an ALT of 169, an ALP of 165 and a bilirubin of 31. Ultrasound showed a thickened irregular CBD but no gall stones and the initial clinical impression was that of having passed a stone. MRCP did not show any calculi, instead there was irregular peripheral duct dilatation in keeping with primary sclerosing cholangitis. She was managed conservatively and was asymptomatic until a second episode a year later. A CT was performed and showed progressive worsening of the ductal disease with CBD dilatation (maximum luminal diameter 9mm). Subsequently a liver biopsy showed hyperplastic and ectatic bile ducts with an associated periportal neutrophilic infiltrate, fibrosis and evidence of cholestasis; features consistent with CHF. No Mallory's hyaline, alpha – 1 antitrypsin bodies or HBsA positivity were seen. CHF is a challenging diagnosis to make and was particularly so in this unusual presentation and at this age. Liver biopsy was the definitive investigation in this case. CHF is a chronic disease which is often a challenge to manage, curative only by transplantation.

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