Winter Meeting Programme

7–8 January 2010

6 January 2010 – Satellite Meeting

6 January 2010
Satellite Meeting
Tissue Banking in the NHS – the advantages for Pathology Departments
Organised by the Wales Cancer Bank
Visit **www.pathsoc.org** for programme and registration

❁

7–8 January 2010
Winter Meeting (197th Scientific Meeting)

❁

Hosted
by the Department of Histopathology
Imperial College, London

❁

Venue
Imperial College, South Kensington Campus,
Sir Alexander Fleming Building, Imperial College Road,
London SW7 2AZ

Contents

Programme Quick Reference Tables

THURSDAY 7 JANUARY

Foyer (Ground Floor)

08.00	Registration and Coffee

Room G28 – Computer Lab (Ground Floor) — *viewing via PCs*
Room G64 (Ground Floor) — *viewing via microscopes*

09.00–15.00	Slide Seminar Competition and Viewing: *Hammersmith black box cases*
15.00–17.30	Slide Seminar Viewing only (competition closes at 15.00)

Room G16 – Lecture Theatre (First Floor)

09.15–13.00	Symposium: *Advances in cancer biology*

Foyer (Ground Floor)

11.00–11.45	Coffee and Trade Exhibition

Rooms 119–122 (First Floor)

11.00–11.45	Poster Viewing

Foyer (Ground Floor)

13.00–14.00	Lunch and Trade Exhibition

Room G16 – Lecture Theatre (First Floor)

14.00–16.30	Plenary Oral Presentations
16.30	**Presentation:** Pathological Society Undergraduate Essay Prize: *Presentation to Miss M Stolbrink, Oxford*

Foyer (Ground Floor)

15.15–15.45	Tea

Rooms 119–122 (First Floor)

15.15–15.45	Poster Viewing
16.30–17.30	Poster Viewing and Chairman's Rounds

Room G16 – Lecture Theatre (First Floor)

17.30	**Presentation:** *Journal of Pathology* 1st Jass Prize for Research Excellence in Pathology: *Presentation to Dr U Lehmann, Hanover, Germany*
17.35–18.35	Pathological Society's Goudie Lecture: Dr N Coleman, Cambridge

The Lanesborough Hotel, Hyde Park Corner, London SW1X 7TA

19.30	Society Dinner

Programme Quick Reference Tables

FRIDAY 8 JANUARY

Foyer (Ground Floor)	
07.30	Registration and Coffee

Room G16 – Lecture Theatre (First Floor)

08.00–09.00	Trainees Session – Meet the Experts *The role of the pathologist in sudden cardiac death*, Dr MN Sheppard, London
09.00–10.15	Slide Seminar Review: Hammersmith black box presentations

Room G28 – Computer Lab (Ground Floor) — *viewing via PCs*
Room G64 (Ground Floor) — *viewing via microscopes*

09.00–14.30	Slide Seminar Viewing: *Hammersmith black box cases*

Foyer (Ground Floor)

10.15–10.45	Coffee

Rooms 119–122 (First Floor)

10.15–10.45	Poster Viewing

Room G16 – Lecture Theatre (First Floor)

10.45–12.35	Symposium: *Do you see what I see? – Beyond light microscopy*

Foyer (Ground Floor)

12.35–13.30	Lunch and Trade Exhibition – *including Zeiss virtual microscopy imaging live demonstration (outside the building)*

Rooms 119–122 (First Floor)

13.30–14.30	Poster Viewing and Chairman's Rounds

Room G16 – Lecture Theatre (First Floor)

14.30–16.00	Symposium: *The Lazarus phenomenon – pathologists and academic research*

Scientific Sessions Information

PLENARY ORAL SESSION *Room G16 – Lecture Theatre (First Floor)*

The eight highest-ranked submitted oral abstracts will be presented on Thursday 7 January, 14.00–16.30.

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 *Rooms 119–122 (First Floor)*

Viewing
Thursday 7 January, 11.00–11.45 and 15.15–15.45
Friday 8 January, 10.15–10.45

Chairman's Formal Poster Rounds
Thursday 7 January, 16.30–17.30
Friday 8 January, 13.30–14.30

Prizes
Poster round chairs will be circulating on Thursday 7 January to select the winners of the Pathological Society Sir Alastair Currie Prize and 2nd and 3rd poster prizes. *Due to shortening of the meeting programme, if your poster is not displayed on Thursday 7 January then unfortunately it will not be considered for the prizes.* Winners will be announced at the Society Dinner on Thursday 7 January.

Note to Presenters
Ideally posters should be in place on Thursday 7 January by 11.00 and removed by Friday 8 January, 15.00.

Presentation
The presenting author (or another author) 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 – *Hammersmith Black Box Cases*

Slide Seminar Competition and Viewing
Thursday 7 January, 09.00–15.00 (15.00–17.30 viewing only, the competition closes at 15.00)
Friday 8 January, 09.00–14.30 (viewing only)
    –via PCs *Room G28 – Computer Lab (Ground Floor)*
    –via microscopes *Room G64 (Ground Floor)*

Review Session *Room G16 – Lecture Theatre (First Floor)*
Friday 8 January, 09.00–10.15

Prize
The winner will be announced at the Society Dinner on Thursday 7 January, the prize being a case of champagne *(at the discretion of the winner, by tradition, this is shared amongst those present!)*.

CONTINUING PROFESSIONAL DEVELOPMENT (CPD)

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

Thursday 7 January	Full day = 7 credits	Half day = 4 credits
Friday 8 January	Full day = 6 credits	Half day = 3 credits

General Arrangements / Additional Information

SOCIETY DINNER *Lanesborough Hotel, Hyde Park Corner, London SW1X 7TA*

Thursday 7 January.

Tickets are £55 – please book your ticket(s) when registering on-line.

For information on the Lanesborough Hotel visit: **www.lanesborough.com**

TRADE EXHIBITION *Foyer (Ground Floor)*

There will be a Trade Exhibition adjacent to the catering point.

INTERNET ACCESS *Room G28 – Computer Lab (Ground Floor)*

Delegates will be issued with usernames and passwords at the registration desk. Wireless access will also be available. Delegates whose home institutions are signed up to the *Eduroam* system may access wireless via that account.

MESSAGES

During the meeting, messages for delegates may be left on the following number: **07818 640 887** (mobile).

REFRESHMENTS *Foyer (Ground Floor)*

All refreshments will be served on the Ground floor, Foyer area.

BADGES

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

COATS AND BAGS *Room G60 (Ground Floor)*

Secure facilities will be provided for coats and luggage.

TRAVEL, ACCOMMODATION AND VENUE INFORMATION

Please visit the meeting website for information: **http://asp.artegis.com/2010WinterDelReg**

LOCAL PLACES OF INTEREST

For information please visit: **www.visitlondon.com**

ENQUIRIES

Before the Meeting enquiries should be addressed to:
Pathological Society
Telephone: +44 (0)20 7976 1260
Fax: +44 (0)20 7930 2981
E-mail: **admin@pathsoc.org**

DISCLAIMER

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

Future Meetings

2010

25–29 January   Pathological Society 3rd Winter School for Trainees
The Kensington Close Hotel, London

29 June – 1 July   Summer Meeting, St. Andrews

2011

6–7 January   Winter Meeting, including Trainees' Programme, Cambridge
The Kensington Close Hotel, London

10–13 May   Ghent Pathology 2011
(6th Joint Meeting of the British Division of the IAP and the Pathological Society)

Registration

DELEGATE TYPE	FEE CATEGORIES	DAY or PART DAY		SOCIETY DINNER
		UP TO AND INCLUDING 23 NOV 2009	AFTER 23 NOV 2009	
Pathological Society Members	Ordinary Members, Consultant and/or equivalent position	£ 90	£ 140	£ 55
Pathological Society Concessionary Members	Biomedical Scientists; Honorary or Senior Members; PhD Students; Post-Doctoral Fellows, Technicians and Trainees	£ 30	£ 50	£ 55
Undergraduate Students ✻		£ 30	£ 50	£ 55
Non-Members	Consultant and/or equivalent position	£ 140	£ 200	£ 55
Non-Members Concessionary ✻	Biomedical Scientists; PhD Students; Post-Doctoral Fellows, Technicians and Trainees	£ 45	£ 70	£ 55

REGISTRATION FEES — FEES INCLUDE REFRESHMENTS AND LUNCH

ADVANCE REGISTRATION

Registration is via our on-line facility found on our website: **www.pathsoc.org**
Advance registration will close on Monday 14 December 2009.
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.
Please e-mail to: **julie@pathsoc.org**

CANCELLATIONS

Please note that we are unable to refund registration fees for cancellations received after Friday 11 December 2009.

DELEGATE ENROLMENT (AT THE MEETING)

Enrolment at the Delegate Reception Desk will take place from 08.00 hrs.

Detailed Programme – Thursday 7 January 2010

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

08.00 **The Foyer (Ground Floor)**
REGISTRATION and COFFEE

09.00–15.00 **Room G28 – Computer Lab (Ground Floor)** — *viewing via PCs*
Room G64 (Ground Floor) — *viewing via microscopes*
SLIDE SEMINAR COMPETITION and VIEWING: *The Hammersmith black box cases*
15.00–17.30 Slide Seminar Viewing only (competition closes at 15.00)

09.15–13.00 **Room G16 – Lecture Theatre (First Floor)**
SYMPOSIUM: *Advances in cancer biology*
Chair: Prof G Stamp, Imperial College, London

09.15–09.50 **[S13]** *The future of cancer care*
Prof K Sikora, CancerPartners UK, London

09.50–10.25 **[S1]** *Inflammation and the cancer microenvironment*
Prof F Balkwill, Institute of Cancer, Barts and the London School of Medicine
and Dentistry

10.25–11.00 **[S2]** *Cancer genetics*
Prof I Tomlinson, University of Oxford

11.00–11.45 **COFFEE and TRADE EXHIBITION** — **The Foyer (Ground Floor)**
POSTER VIEWING — **Rooms 119–122 (First Floor)**

11.45–12.20 **[S3]** *Evolution of the Cancer Genome*
Prof M Stratton, The Wellcome Trust Sanger Institute, Cambridge

12.20–13.00 *Cancer diagnostics and personalised medicine*
Prof D Sidranksy, Director Head and Neck Cancer Research, Johns Hopkins University
School of Medicine, Baltimore, USA

13.00–14.00 **The Foyer (Ground Floor)**
LUNCH and TRADE EXHIBITION

14.00–16.30 **Room G16 – Lecture Theatre (First Floor)**
PLENARY ORAL PRESENTATIONS
Chair: Prof IO Ellis, University of Nottingham
Prof G Stamp, Imperial College, London

14.00–14.15 **[PL1]** *Molecular heterogeneity within G3 breast cancer*
{P} K Unger, S Oliveros, H Zitzlesberger, S Riley, C Davies, DW Williams, M Gudi,
RCF Leonard, GA Thomas

14.15–14.30 **[PL2]** *Refining the diagnosis and EGFR status of non-small cell carcinoma in biopsy and
cytologic material, using a panel of mucin staining, TTF-1, Cytokeratin 5/6 and P63, and
EGFR Mutation Analysis*
{P} M Deshmukh, D Gonzalez, P Shah, M Pynegar, A Rice, S Popat, A Nicholson

---

| 14.30–14.45 | **[PL3]** | *Novel potential therapeutic targets for cholangiocarcinoma identified by array comparative genomic hybridization*
{P} S McKay, K Unger, D Spalding, R Hutchins, G Thomas, G Stamp |

| 14.45–15.00 | **[PL4]** | *Comparative genomic hybridisation analysis of the origin of pseudomyxoma peritonei*
{P} H El-Daly, G Poulogiannis, R Hamoudi, MJ Arends |

| 15.00–15.15 | **[PL5]** | *Ewing's sarcoma cells express RANKL and MCSF and promote osteoclast formation*
{P} R Taylor, F Hemingway, H Knowles, NA Athanasou |

15.15–15.45 TEA — The Foyer (Ground Floor)
POSTER VIEWING — Rooms 119–122 (First Floor)

| 15.45–16.00 | **[PL6]** | *Expression of Interleukin-8 (IL-8), IL-8RA and IL-8RB in ovarian carcinoma and the effect on cisplatin resistance*
R Aiyappa, E Stronach, H Gabra, {P} M El-Bahrawy |

| 16.00–16.15 | **[PL7]** | *Malignant germ cell tumours display common microRNA profiles resulting in global changes in expression of mRNA targets*
{P} M Murray, H Saini, S Van Dongen, C Abreu-Goodger, R Palmer, J Nicholson, A Enright, N Coleman |

| 16.15–16.30 | **[PL8]** | *A new 3D microscopy method for enhancing morphological diagnosis and mapping the 'Chromatome'*
{P} PJ Tadrous |

16.30   Room G16 – Lecture Theatre (First Floor)
PRESENTATION: 2009 Pathological Society's Undergraduate Essay Competition
Prize Winner: Miss M Stolbrink, Oxford

16.30–17.30   Rooms 119–122 (First Floor)
POSTER VIEWING and CHAIRMAN'S ROUNDS

CATEGORIES	POSTER NUMBERS
Cellular/Molecular Pathology	P 1–P11[1]
Experimental Tumour Pathology	P12[1]
Endocrine	P13 [2]
Head & Neck	P14[2]
Neuropathology/Ophthalmic	P15[2]
Osteoarticular/Soft Tissue	P16–P19[2]
Skin	P20–P22[2]
Gastrointestinal	P23–P29[3]
Hepatobiliary/Pancreas	P30–P34[3]
Genitourinary/Renal	P35–P40[4]
Gynaecological	P41–P42[4]
Lymphoreticular	P43–P47[5]

Chair: [1] Dr MJ Arends, Cambridge and Dr JS Reis-Filho, London
[2] Dr N Francis, London and Dr F Roncaroli, London
[3] Dr P Cohen, London and Dr R Goldin, London
[4] Dr M El-Bahrawy, London and Prof JE Martin, London
[5] Dr K Naresh, London and Dr EJ Soilleux , Oxford

17.30   **Room G16 – Lecture Theatre (First Floor)**
PRESENTATION: *Journal of Pathology* – **1st Jeremy Jass Prize for Excellence in Pathology**
Prize Winner: Dr U Lehmann, Hanover, Germany

17.35–18.35   **Room G16 – Lecture Theatre (First Floor)**
THE PATHOLOGICAL SOCIETY OF GREAT BRITAIN & IRELAND'S
6th GOUDIE LECTURE
Chair: Prof CS Herrington, General Secretary, Pathological Society

[S4]   *Viral and host contributions to HPV–associated squamous carcinogenesis: prospects for improved clinical management*
Dr N Coleman, Hutchison/MRC Research Centre, Cambridge

19.30   **The Lanesborough Hotel, London SW1**
SOCIETY DINNER

07.30   **The Foyer (Ground Floor)**
REGISTRATION and COFFEE

08.00–09.00   **Room G16 – Lecture Theatre (First Floor)**
TRAINEES SESSION – MEET THE EXPERTS
Chair: Dr I Proctor, University College London

[S5]   *The role of the pathologist in sudden cardiac death*
Dr MN Sheppard, Royal Brompton Hospital London and CRY Cardiac Pathology
Laboratory, Imperial College, London

09.00–10.15   **Room G16 – Lecture Theatre (First Floor)**
SLIDE SEMINAR REVIEW: *Hammersmith black box cases*
Chair and Presenters:
Dr M El-Bahrawy, Dr N Francis, Dr M Gudi, Dr K Naresh, Dr F Roncaroli
– all at Imperial College, London

09.00–14.30   **Room G28 – Computer Lab (Ground Floor)** — *viewing via PCs*
Room G64 (Ground Floor) — *viewing via microscopes*
SLIDE SEMINAR VIEWING: *Hammersmith black box cases*

10.15-10.45   **COFFEE and TRADE EXHIBITION** — The Foyer (Ground Floor)
POSTER VIEWING — Rooms 119–122 (First Floor)

10.45–12.35   **Room G16 – Lecture Theatre (First Floor)**
SYMPOSIUM: *Do you see what I see? – Beyond light microscopy*
Chair: Dr C Dunsby, Imperial College, London

10.45–11.25   **[S6]**   *Multi-dimensional fluorescence imaging – for high content and/or label-free bioimaging*
Prof PMW French, Imperial College, London

11.25–12.00   **[S7]**   *Multi-dimensional ultrastructure*
Dr L Collinson, Cancer Research UK, London Research Institute

12.00–12.35   **[S8]**   *Pathologist-free microscopy?*
Prof R Bhargava, University of Illinois at Urbana-Champaign, USA

12.35–13.30   **The Foyer (Ground Floor)**
LUNCH and TRADE EXHIBITION*
* including Zeiss Virtual Microscopy Imaging
– *live demonstration (outside the building entrance)*

13.30–14.30 **Rooms 119–122 (First Floor)**
POSTER VIEWING and CHAIRMAN'S ROUNDS

CATEGORIES	POSTER NUMBERS
Autopsy & Forensic	P48–P52[1]
Education & Audit	P53–P59[1]
Breast	P60–P70[2]
Cardiovascular/Pulmonary	P71–P83[3]
Technical Advances	P84–P93[4]

Chair: [1] Prof S Lucas, London and Dr J Wang, London
[2] Dr S Shousha, London and Prof RA Walker, Leicester
[3] Dr J Boyle, London and Dr MN Sheppard, London
[4] Dr PJ Tadrous, Harrow and Dr SS Cross, Sheffield

14.30–16.00 **Room G16 – Lecture Theatre (First Floor)**
SYMPOSIUM: *The Lazarus phenomenon – pathologists in academic research*
Chair: Prof GA Thomas, Imperial College, London

14.30–14.50 **[S9]** *Developmental phenotyping: combining traditional pathology and cutting edge technologies*
Dr B Spencer-Dene, Imperial College, London and The London Research Institute – CRUK

14.50–15.10 **[S10]** *Translational clinical trials*
Prof SE Pinder, King's College London, Research Breast Pathology, Guy's Hospital

15.10–15.30 **[S11]** *Fixed forever? Fixation and molecular techniques*
Prof GA Thomas, Imperial College London

15.30–16.00 **[S12]** *Tissue specimens within the paradigm of 'fit for purpose'*
Prof S Hewitt, National Cancer Institute, National Institutes of Health, Bethesda, USA

16.00 Thanks and farewell
Prof GW Stamp and Prof GA Thomas

Acknowledgments (Trade Exhibition)

**The Pathological Society of Great Britain & Ireland
wishes to acknowledge the support of the following companies
participating in the TRADE EXHIBITION**

CARL ZEISS UK LTD

Preparation, Visualisation, and Analysis, digital pathology requires the integrated workflow provided by Carl Zeiss. Come to the Carl Zeiss trade stand to see the latest developments in this exciting area and for your chance to win a Carl Zeiss Logitech webcam. Carl Zeiss will be launching its brand new automated TMA preparation system.

GATAN UK LTD

A new 3D microscopy technique has been developed by Gatan into a commercial instrument, following initial work by Professor Winfried Denk at the Max Planck Institute in Heidleberg, Germany. The Gatan '3View' system uses the technique of Serial Block-Face Scanning Electron Microscopy (SBFSEM) to acquire aligned serial images of a fresh-cut block of embedded tissue. A typical experiment will result in up to 1000 images of the tissue at high resolution. The Gatan 3View allows biological features to be visualised in three dimensions and understood in a manner that has up to now been impossible to achieve in practice.

i-PATH DIAGNOSTICS LTD

i-Path Diagnostics Ltd is a Northern Ireland based company that has grown out of a leading academic research group within Queen's University Belfast.

i-Path has developed a suite of novel software products including InView™; e-Learning materials and high quality educational content in pathology based on virtual microscopy and PathXL™; an on-line content management and authoring platform for a range of virtual microscopy applications, which can include PathXL OLT, an online testing wizard ideal for delivering cost effective student testing.

i-Path's products facilitate:
- ➢ Web-based education/training of pathology
- ➢ Online pathology testing
- ➢ Consultation networks
- ➢ Multi-disciplinary teams
- ➢ Tissue-banking/archiving

MENARINI DIAGNOSTICS

Menarini is one of the fastest growing Diagnostics companies in the UK. As a leading supplier of products for immunohistochemistry, Menarini Diagnostics is committed to providing technologically advanced solutions for cellular pathology laboratories. Menarini is also proud to announce the launch of the D-Sight, the most recent innovation in Virtual Microscopy and Image Analysis Systems.

WILEY-BLACKWELL

Journal of Pathology

Collect your FREE COPY of the *Journal of Pathology* and our Annual Review Issue on Stem Cells in Pathobiology and Regenerative Medicine from the Wiley-Blackwell stand and hear about our special trainee-member subscription rates.

A leading pathology journal, the *Journal of Pathology* serves as a bridge between basic biomedical science and clinical medicine with particular emphasis on morphologically based studies. The main interests of the Journal lie in understanding the pathophysiological and pathogenetic mechanisms of human disease.

Find out how to submit your next paper to the *Journal of Pathology* at the Wiley-Blackwell stand.

Acknowledgments (Trade Exhibition)

The Pathological Society of Great Britain & Ireland
wishes to acknowledge the support of the following companies
participating in the TRADE EXHIBITION

WISEPRESS.COM

Europe's leading conference bookseller, has a complete range of books and journals relevant to the themes of the meeting. Books can be purchased at the stand or, if you would rather not carry them, posted to you – Wisepress will deliver worldwide. In addition to attending 250 conferences per year, Wisepress has a comprehensive medical and scientific bookshop online with great offers, some up to 40% off the publisher list prices.

Wisepress Online Bookshop
Address: The Old Lamp Works, 25 High Path, Merton Abbey, London SW19 2JL, UK
Phone: +44 20 8715 1812
Fax: +44 20 8715 1722
Email: **bookshop@wisepress.com**
Website: **www.wisepress.com**

Abstracts

Plenary

Note: Presenter's name is shown in **bold**

PL1

Molecular heterogeneity within G3 breast cancer

K Unger[1], S Oliveros[1], H Zitzlesberger[2], S Riley[3], C Davies[3], DW Williams[3], M Gudi[1], RCF Leonard[1], GA Thomas[1]
[1]*Imperial College London, [2]Helmholtz Zentrum Munich, [3]Singleton Hospital, Swansea*

The aim of this study was to examine copy number alteration (CNA) in Grade 3 (G3) breast cancer and its relationship to nodal status (N-ve/N+ve), clinical outcome, and age at diagnosis and to seek genetic markers of prognosis for treatment tailoring in this group. DNA was extracted from sections from 81 cases operated at Singleton Hospital and genomic CNA were identified using 1Mb BAC array CGH. Unsupervised hierarchical clustering, supervised, correlation and survival analyses were carried out using packages and tests within the R statistical platform. The analysis showed significant copy number gains on chromosomes 1,4,5,7,11 and 21 in N+ve and losses on chromosomes 1,6 and 10 in N-ve G3. One region of chromosome 1 (1p36 – 1p35) in particular was lost in N0 but gained in N+ve, and this was significantly associated with ER-ve/N+ve cases (FDR p-value < 0.05). BAC aCGH profiles did not significantly associate with ER status in G3 disease. Two clusters, delineated on unsupervised hierarchical cluster analysis, correlated with overall survival. Neither correlated with nodal or ER status, indicating that further analysis may discover genes of prognostic potential within these profiles. When solely N0 cases from either young premenopausal or older postmenopausal women were analysed, three distinct groups were identified with distinct changes in small regions on chromosomes 1, 9, 10, 14 and 20. Further dissection of these differences may lead to better tailored treatment for poor prognosis breast cancer.

PL2

Refining the diagnosis and EGFR status of non-small cell carcinoma in biopsy and cytologic material, using a panel of mucin staining, TTF-1, Cytokeratin 5/6 and P63, and EGFR Mutation Analysis

M Deshmukh[1], D Gonzalez[2], P Shah[3], M Pynegar[1], A Rice[1], S Popat[4], A Nicholson[1]
[1]*Department of Histopathology, Royal Brompton Hospital, London*
[2]*Molecular Diagnostics, The Institute of Cancer Research, Surrey*
[3]*Department of Respiratory Medicine, Royal Brompton Hospital, London*
[4]*Department of Medical Oncology, Royal Marsden Hospital, London and National Heart and Lung Institute, Imperial College, London*

The dichotomization of non-small cell carcinoma (NSCLC) into squamous (SQCC) and adenocarcinoma (ADC) is increasingly required with regard to oncological management. This study determines the utility of an antibody panel in refining the diagnosis on small biopsies and cytological samples, and whether such material is suitable for somatic EGFR genotyping.
32 consecutive cases of NSCLC were first tested using a panel comprising CK5/6, P63, TTF-1, 34βE12 and a D-PAS stain for mucin, to determine their value in refining diagnosis of NSCLC. Following this test phase, two further pathologists independently reviewed the cases using a refined panel that excluded 34βE12 due to its low specificity for SQCC, and refinement of diagnosis and concordance were assessed. 10 cases of adenocarcinoma, including 8 derived from cytologic samples, were sent for EGFR mutation analysis.
There was refinement of diagnosis in 65% of cases of NSCLC to either SQCC or ADC in the test phase. This included 10 of 13 cases where cell pellets had been prepared from transbronchial needle aspirates (TBNAs). Validation by two further pathologists confirmed increased refinement and concordance of diagnosis. All samples were adequate for analysis and all showed a wild-type EGFR genotype.
A panel comprising CK5/6, P63, TTF-1 and a D-PAS stain increases diagnostic accuracy and agreement between pathologists when faced with refining a diagnosis of NSCLC to SQCC or ADC. These small samples, even cell pellets derived from TBNAs, appear adequate for EGFR mutation analysis.

PL3

Novel potential therapeutic targets for cholangiocarcinoma identified by array comparative genomic hybridization

S McKay[1], K Unger[1], D Spalding[1], R Hutchins[2], G Thomas[1], G Stamp[1]
[1]*Imperial College London, [2]Barts and the London Hospital*

Cholangiocarcinoma is a rare tumour with a poor prognosis. We evaluated DNA copy number alterations between intrahepatic (ICC), perihilar (PHCC), and extrahepatic (ECC) cholangiocarcinoma (CC), to identify novel potential therapeutic targets. 22 cases were analysed (7 ICC, 5 PHCC, 9 ECC) using DNA extracted from sections of FFPE tissue. BAC array CGH was performed to identify copy number alterations (CNA). CNAs were least frequent among ECC. Gains at 17q12, 17p13.3-q21.33, 17q22-q25.3, and 22q11.1-q13.3 were found in all cases and differing profiles were found between tumours from different anatomical sites. ICC and PHCC showed common regions of CNA at 11q12.2-q13.4, 19p13.11-p13.3, 19q13.11-q13.43. The common alterations seen on 17q12, 11q12.2-q13.4, which represent potential amplification of the Her2 and Vegfb genes, respectively suggest that agents targeted to these two factors may be clinically useful in some CC. A lower frequency of gain of 7p11.2 (42.9% ICC and 20% PHCC) which harbours the EGFR gene may also suggest the possible use of EGFR inhibitors. Interestingly, gain of this region was not identified in ECC. This study illustrates the genetic variability of CC and indicates the need for molecular stratification of these patients prior to treatment.

PL4

Comparative genomic hybridisation analysis of the origin of pseudomyxoma peritonei

H El-Daly[1], G Poulogiannis[1], R Hamoudi[1], MJ Arends[1]
[1]*Department of Pathology, University of Cambridge, Cambridge*

Pseudomyxoma peritonei is an uncommon syndrome, with heterogeneous appearances, variable outcomes and uncertain origins. The aim was to use array-comparative genomic hybridisation (aCGH) to determine the genomic changes in neoplastic mucinous epithelium taken from different sites involved by pseudomyxoma peritonei, including appendix, ovary and peritoneum, that may clarify the origin and shed light on disease progression. DNA samples extracted from paraffin embedded blocks of mucinous epithelium from appendix, omentum and ovary from eleven pseudomyxoma peritonei patients were analysed by aCGH using pan-genomic 1MB arrays. DNA copy number alterations (CNA) showed a high frequency of losses detected on chromosomes 1p, 5q, 7q, 10p, 10q, 11q, 14q and 22q. The frequency of CNA gains was lower with gains detected on chromosomes 1p and 1q. Cluster analysis of smoothened array data showed consistent grouping of samples from different sites from the same patient. In one case, samples from the three sites showed amplification of myc oncogene on chromosome 8, as well as progressive amplification of TSPAN8 and P-CIP1/RASSF9 genes on chromosome 12 with lower levels of amplification in the appendiceal neoplasm and higher levels of DNA amplification in the omental and ovarian neoplasms. In conclusion, the data suggest a common origin of pseudomyxoma peritonei deposits in ovary and peritoneum with the appendiceal tumour being the primary site. They also show myc amplification as an early event in some cases, with progressive amplification of cancer-related genes as one mechanism associated with disease progression.

PL5

Ewing's sarcoma cells express RANKL and MCSF and promote osteoclast formation

R Taylor[1], F Hemingway[1], H Knowles[1], NA Athanasou[1]
[1]*University of Oxford*

Ewing's sarcoma is a primary bone tumour associated with extensive bone destruction. To investigate the osteolysis in Ewing's sarcoma we investigated whether Ewing's tumour cells can directly effect matrix resorption and their role in promoting osteoclast differentiation.

Cultures of Ewing's tumour cell lines (A673, RDES, SKES-1, SK-N-MC and TC71) on dentine slices did not result in lacunar resorption or the release of C-terminal telopeptides of type 1 collagen (CTX-1), a product of matrix degradation, assayed by ELISA. Expression of RANKL and MCSF, essential co-factors required for osteoclastogenesis, was identified in Ewing's sarcoma TMAs by immunohistochemistry, and membrane bound RANKL protein was identified in the tumour cells by Western Blotting. Co-cultures of Ewing's cells, particularly SK-N-MC cells, with MCSF primed CD14+ monocytes resulted in the formation of numerous multinucleated cells that expressed the osteoclast markers TRAP and VNR.

Our results indicate that Ewing's tumour cells do not directly effect matrix resorption, but that these cells strongly express MCSF and RANKL, and can support osteoclastogenesis. Consequently, these may partly account for the aggressive bone destruction associated with this tumour. Stimulation of RANKL-dependent osteoclastogenesis could explain the aggressive and extensive osteolysis of Ewing's sarcoma and may provide a potential pathway for therapeutic intervention.

PL6

Expression of Interleukin-8 (IL-8), IL-8RA and IL-8RB in ovarian carcinoma and the effect on cisplatin resistance

R Aiyappa[1], E Stronach[2], H Gabra[2], M El-Bahrawy[1]
[1]*Department of Histopathology, Division of Investigative Science, Imperial College, London, United Kingdom, [2]Ovarian Cancer Action (HHMT) Research Centre and Section of Molecular Therapeutics, Division of Surgery, Oncology, Reproductive Biology and Anaesthetics, Imperial College, London, United Kingdom*

This study investigates the role of the cytokine IL-8 and IL-8 receptors, IL-8RA and IL-8RB in conferring platinum resistance in ovarian carcinoma.
We studied the expression of IL-8 and IL-8 receptors in isogenically matched platinum sensitive (PEO1, PEA1, PEO14) and resistant (PEO4, PEA2, PEO23) ovarian cancer cell lines and ovarian tumours. We investigated the effect siRNA knockdown of IL-8 and IL-8 receptors in resistant PEO4 cells.
qRT-PCR and immunoflourescence showed all lines expressed IL-8 and IL-8 receptors with resistant lines expressing higher receptor levels as compared to sensitive lines; IL-RA being the receptor more strongly expressed. Cisplatin treatment increased expression of IL-8 and IL-8 receptors and induced nuclear localisation of IL-8RA.
Immunostaining ovarian tumours confirmed invitro findings, with platinum resistant tumours showing more frequent and higher levels of receptor expression than sensitive tumours. IL-RA was the receptor more frequently and strongly expressed (72%) as compared to IL-RB (55%) and was observed in the nuclei of only resistant tumours.
siRNA knockdown of IL-8 and IL-8 receptors did not re-sensitise the resistant PEO4 cells to cisplatin.
We conclude that upregulation of IL-8 and IL-8 receptors does not induce platinum resistance in ovarian cancer. However, cisplatin upregulates IL- 8 and IL-8 receptor expression and induces nuclear localisation of IL-8RA, suggesting they have a role in tumour progression and are potential therapeutic targets in ovarian tumours, particularly platinum resistant tumours.

PL7

Malignant germ cell tumours display common microRNA profiles resulting in global changes in expression of mRNA targets

M Murray[1], H Saini[2], S Van Dongen[2], C Abreu-Goodger[2], R Palmer[1], J Nicholson[3], A Enright[2], N Coleman[1-4]
[1]*MRC Cancer Cell Unit, Cambridge, UK, [2]EMBL-European Bioinformatics Institute, Hinxton, Cambridge, UK, [3]Department of Paediatric Oncology, Addenbrooke's Hospital, Cambridge, UK [4]Department of Pathology, University of Cambridge, UK*

As malignant germ cell tumours (mGCTs) are thought to have a common origin from primordial germ cells, they may share fundamental biological abnormalities despite their clinical and histopathological heterogeneity. We tested the hypothesis that mGCTs are characterized by common patterns of microRNA expression that are functionally significant by affecting levels of mRNA targets.

We used the miRCURY microarray (Exiqon) to quantify global microRNA expression levels in 28 GCTs and 14 nonmalignant samples (eight controls, six teratomas) from paediatric subjects. For 21 of these samples (17 mGCT, four nonmalignant) global mRNA expression profiles had been obtained using the U133A GeneChip (Affymetrix). We compared our findings with a re-analysis of published qRT-PCR microRNA and U133A mRNA profiling in adult mGCTs. Data were analysed using R and Bioconductor. We used the novel bioinformatic algorithm Sylamer to investigate enrichment and depletion of miRNA seed complementary regions (SCRs) in 3'UTRs of mRNAs ranked according to expression levels in mGCTs.

miR-302 and miR-371~373 clusters were universally overexpressed in mGCTs regardless of patient age, tumour histological type or site. Sylamer demonstrated that the most significantly overrepresented SCR in the 3'UTRs of downregulated mRNAs was GCACTT, corresponding to the AAGUGC seed common to miR-302a~d and miR-372~373. Gene ontology analysis showed that the down-regulated mRNAs containing this common SCR in paediatric mGCTs were members of major cancer-associated cellular pathways.

Our data suggests a fundamental role for miR-302 and miR-371~373 clusters in the biology of mGCTs.

PL8

A new 3D microscopy method for enhancing morphological diagnosis and mapping the 'Chromatome'

PJ Tadrous[1]
[1]*Cellular Pathology Dept, Northwick Park Hospital, London*

Background: Brightfield 3D microscopy at the limit of resolution of light microscopy has not previously been realised. This work presents results of a novel method of 3D microscopy for widefield brightfield microscopy of histological samples that provides unprecedented detail of nuclear and cytoplasmic structure in full 3D.
Method: The method uses a form of deconvolution, recently published by the author, where a through-focus Z-stack of the test object is deconvolved using a point-spread function calculated from a stack taken through a thin probe. The deconvolved volume is then visualised by custom 3D software that allows warping to fit a standardised model for comparisons with other test objects. This method was applied to common cell types taken from a haematoxylin-stained thick section of human liver. This paper concentrates on human nuclear chromatin morphology.
Results: The resulting 3D images of chromatin structure of the interphase nucleus are presented for the first time. Connectivity of chromatin in 3D is displayed. The effects of 3D visualisation on simplification of structural interpretation is also illustrated as is the 3D basis for morphological recognition of special cell types.
Discussion: From these models I discuss insights into the nature of morphological interpretation and recognition of cell types from histological preparations and the possibilities of combining the method with in-situ hybridisation to produce molecular maps of the interphase nucleus (termed the 3D 'chromatome' by way of analogy to the 'genome') that could help us understand the mechanisms of genetic and epigenetic interactions.

Abstracts

Poster

Note: Presenter's name is shown in **bold**

P1

Bmi-1 gene silencing causes BMP pathway activation and deregulation of cell adhesion in medulloblastoma cell lines

A Merve[1], X Zhang[1], S Marino[1]
[1]Blizard Institute of Cell and Molecular Science, QMUL, London

Introduction: Medulloblastoma is an aggressive childhood CNS tumour thought to arise from cerebellar granule cell progenitors (GCP). Bmi1 is a polycomb group gene known to play a crucial role in the control of proliferation of GCP. Bone Morphogenic Proteins (BMPs) belong to the TGF-β superfamily and they are shown to inhibit medulloblastoma growth in mouse models.
Aim: To study whether Bmi1 expression in medulloblastoma affects the BMP pathway.
Methods: Bmi1 was downregulated using short sequence silencing RNA (ShRNA) in two human medulloblastoma cell lines DAOY and D458 known to overexpress Bmi1. Immunolabelling for phosphorylated SMAD proteins (pSMAD) which are downstream effectors of the BMP pathway was carried out.
Results: We observed an increase in number of cells expressing pSMAD and increased in number of multicellular aggregates following Bmi1 downregulation when compared to the controls.

Table 1: Labelling index of pSMAD expressing cells

	Bmi1 downregulated cells	Control cells	Student t-test (p)
DAOY	72.85%	57.51%	0.03
D458	83.93%	75.69%	0.01

Table 2: Number of multicelluar aggregates per high power field

	Bmi1 downregulated cells	Control cells	Student t-test (p)
DAOY	2.36	0.55	0.006
D458	4.78	1.61	0.016

Conclusion: Our data suggest that Bmi1 may affect cell adhesion in medulloblastoma cell lines through BMP pathway inhibition.

P2

An investigation of the mechanisms underlying Matrix Metalloproteinase-3 Secretion in human central nervous system tuberculosis

S Dholakia[1], JA Green[1], JS Friedland[1]
[1]Imperial College London

Introduction: Central nervous system (CNS) involvement is the most serious manifestation of extra pulmonary tuberculosis (TB). Matrix metalloproteinases (MMPs) are up-regulated in CNS TB and are implicated in breakdown of the blood brain barrier. The inflammatory phenotype of microglia, resident CNS macrophages, is distinct from that of infiltrating macrophages. The transcription factor NFκB has a central role in the up-regulation of many inflammatory genes, thus we hypothesised that NFκB differentially controls secretion of MMP-3 and its specific tissue inhibitor 1 (TIMP-1).
Methods: Human microglial cells were stimulated with conditioned media from Mycobacterium tuberculosis (M. tb) infected human monocytes (CoMTB). Cells were pre-incubated with NFκB blocking chemicals and secretion of MMP-3 and TIMP-1 were measured by ELISA.
Results: CoMTB increased MMP-3 secretion 5-fold compared to controls. The secretion of TIMP-1 remains unchanged in the presence of CoMTB. Blockade of the NFκB p65 subunit through IKK-2 inhibition demonstrated dose-dependent decreases in the secretion of MMP-3 with no associated change in the expression of TIMP-1. Blockade of NFκB p50 subunit did not show a significant dose dependant decrease.
Discussion: M. tb driven monocyte networks up-regulate MMP-3 secretion in human microglia. NFκB activity appears to be an early step in this process. Involvement of the p65 subunit homodimer is more important in affecting gene expression of MMP-3 than the p50 subunit. Better understanding of the mechanisms involved in CNS TB tissue degradation could identify novel therapeutic targets.

P3

Effects of antimicrobials on the proteome of in vitro models

W Mathieson[1], S Kirkland[1], RCF Leonard[1], GA Thomas[1]
[1]Imperial College London

Cell culture is a widely used technique to study the pathological effects of stress conditions on human cells, by comparing gene/protein expression in cells grown in the presence or absence of toxic compounds. The clinical value of such experiments is dependent on how closely the cell culture conditions represent those found in vivo. Despite this requirement to minimise culture-induced molecular alterations, antibiotics and anti-fungal agents are routinely added to culture medium. Although there is a general acknowledgement that these compounds probably have inherent stress-inducing properties, little is known as to whether the effects are detectible at the proteomic level. The aim of this study was to address whether penicillin/streptomycin and/or amphrotericin B induced significant alterations at the proteomic level. A breast cancer cell line (MCF7) was cultured in the presence/absence of antibiotics and/or Amphotericin B, the cells lysed and their soluble proteomes compared using two-dimensional Difference Gel Electrophoresis. Differentially expressed proteins were excised from the gel, digested in trypsin and identified using tandem mass spectrometry. Members of the protein families involved in protein turnover and stress response were differentially expressed in the presence of antimicrobials. Our results demonstrate that the effects of the use of different microbials in cell culture experiments should be taken into consideration when comparing data from treated cell lines, and that information on the antimicrobials used should always be cited in research papers.

P4

Long term stability of frozen tissue for use in molecular studies

K Unger[1], W Mathieson[1], T Bogdanova[2], V Pushkariev[2], T Myshunina[2], M Tronko[2], GA Thomas[1]
[1]Imperial College London, [2]Institute of Endocrinology and Metabolism, Kiev

Human tissue is a precious resource, and long term storage of material in dedicated tissue banks will be needed to link the molecular profile of cancer at operation to clinical outcome. Some reports have suggested that the quality of samples deteriorates over time. However, there is little evidence base for these conclusions. The Chernobyl Tissue Bank (CTB) has been collecting and storing frozen samples to strict standard protocols from thyroid cancer and adenoma since October 1998. 250 paired samples of normal and neoplastic tissue, provided by the IEM in Kiev, were recently extracted at Imperial College, the Coordinating Centre for the CTB, for use in a number of research projects. The samples had been stored for various times prior to extraction ranging from more than 10 years to a few months. RNA and DNA are extracted from the same tissue block and the quality of RNA assessed using an Agilent Bioanalyser. The median RIN was 7.8 (2.6-9.4) and there was no effect of the time in storage on the RIN, with samples stored for less than 2 years (n=23) giving a mean RIN of 7.8 ±1.3, and those stored for more than 9 years (n=18) a RIN of 7.4 ± 0.8. These results suggest that storage of frozen tissues in -80C freezers does not result in significant degradation of intact RNA species over a 10 year period.

P5

The Wales Cancer Bank: engaging NHS pathologists in research tissue banking

G Thomas[1], A Parry-Jones[2], M Mason[1]
[1]*Velindre Hospital, Cardiff,* [2]*Cardiff University*

The Wales Cancer Bank (WCB) is a Welsh Assembly Government and Cancer Research Wales funded initiative that aims to collect blood, serum and frozen and FFPE tissue from all patients undergoing treatment for cancer in Wales. Established in 2004, the WCB has since consented over 3202 patients. The current collection includes 17 tissue sites, the largest collections being breast, colorectal, prostate, bladder, renal, head and neck and ovarian. Data is linked-anonymised via the all-Wales electronic clinical database (CanISC), and consent permits future access to clinical outcome data. WCB is licensed by the Human Tissue Authority and approved by NRES as a Research Tissue Bank, obviating the need for researchers using material from the bank to apply to NRES for approval of their research projects. Currently, WCB holds or has access to tissue from 3000 patients, and has received applications from 44 research projects from within and outside the UK. Input from pathologists in Wales was crucial to the development of the bank and much of the funds provided for the bank supports staff and resources in Histopathology departments in Wales. To maximise potential, processed and quality assured materials are provided to researchers. Biomedical scientists are involved in DNA and RNA extraction from frozen and FFPE tissue and the WCB supports a number of MSc projects for biomedical scientists. The project seeks to establish molecular techniques within Pathology Departments in Wales and demonstrates the importance of engagement of NHS based pathologists in provision and use of tissue for research.

P6

Cancer-Associated Fibroblasts from pancreatic cancers have genome copy number changes

J Wang[1], N Johnson[1], K Unger[1], R Ahmad[1], M Nohadani[1], G Thomas[1], G Stamp[1]
[1]*Department of Histopathology, Imperial College London*

Introduction: Cancer progression is not determined by the neoplastic cells alone, but is dependent on the microenvironment or stroma, the dominant population of which is the activated cancer-associated fibroblast (CAF). Recent evidence suggests that stromal elements may harbour mutations or chromosomal changes in a variety of different cancers. In this study, we apply Bacterial Artificial Chromosome (BAC) array comparative genomic hybridisation (arrayCGH) to primary CAF cell lines to determine their changes in genomic copy number.
Methods: Fresh pancreatic cancer resection specimens were collected at pancreatectomy. Isolation and growth of CAF cell lines from tissues at the tumour edge were performed using the outgrowth method. DNA extracted from the cell lines and reference DNA were differentially labelled with Cy3 and Cy5, co-hybridised onto 1 Mb BAC arrays, scanned and analysed for chromosomal gains and losses.
Results: 5 CAF cell lines have been obtained from the edge of pancreatic ductal adenocarcinomas. The purity of the cell population was confirmed by immunofluorescence for □SMA, desmin and FSP1. ArrayCGH analyses to date of 3 of the cell lines show no chromosome losses in the 3 cell lines. However, regions of chromosome gain were detected at chromosome arms 6p, 7q and 11q.
Conclusions: We show that CAFs from pancreatic cancers have multiple areas of chromosome copy number changes using arrayCGH. Further studies are warranted, including the use of arrayCGH for laser-microdissected stromal tissues and gene mutation analyses.

P7

Array comparative hybridisation to cholangiocarcinoma cell lines HuCC-T1 and SkChA-1

S Pericleous[1], SC McKay[1], W Mathieson[1], K Unger[1], DR Spalding[1], RR Hutchins[2], G Thomas[1], G Stamp[1]
[1]*Imperial College London,* [2]*The Royal London Hospital*

Introduction: Cholangiocarcinoma (CC) has a dismal prognosis. Few cell lines and disease models exist for in vitro experimentation. We used array comparative genomic hybridisation (arrayCGH) to determine DNA copy number alterations (CNAs) in two established CC cell lines.
Methods: Sk-ChA-1 (extrahepatic) and HUCC-T1 (intrahepatic) CC cell lines were used. ArrayCGH was performed using 1Mb BAC arrays. Analysis was done using CAPweb.
Results: CNAs common to both cell line included gains of 7p/q, and 14q21.3-32.33, losses at 1p33-36.3 and 9q33.2-34.4. CNAs unique to Sk-ChA-1 included gains at 1p11.2-32.3, 2p21-16.3, and losses at 17p13.36-11.2, and 6p22.3. Gains unique to HUCC-T1 included 5q23.2-p15.33, 11p14.2-q25, and losses at 6p/q, 13q, and 18p/q.
Conclusions: In this study we employed array CGH to analyse two of the most widely used cholangiocarcinoma cell lines. These cell lines demonstrated some common CNAs, however many more CNAs unique to each cell line were identified. This analysis aims to identify the most appropriate cell line in relation to primary tumour characteristics to determine their suitability as in vitro disease models.

P8

The role of LKB1 and the mTOR Pathway in osteosarcoma pathogenesis

L Duhamel[1], A Shalaby[1], H Ye[2], D Halai[1], B Idowu[1], N Presneau[1], AM Flanagan[1, 2]
[1]*Department of Pathology, Cancer Institute, University College London*
[2]*Department of Histopathology, Institute of Orthopaedics & Musculoskeletal Science, The Royal National Orthopaedic Hospital, Stanmore*

Osteosarcoma (OS) is a rare cancer affecting approximately 200 persons per annum in the UK. Neo-adjuvant chemotherapy increased the 5-year survival from 20% to 50% in the early 1980s but it has now stabilised at approximately 60%. New treatments are required if the survival of patient with this disease is to increase. It has been reported that mice deficient in one LKB1 allele, a tumour suppressor gene associated with Peutz-Jeuger syndrome, develop osteoblastic tumours. LKB1 represses activation of the mammalian target of rapamycin (mTOR) pathway, which has become a promising therapeutic target in the treatment of cancer. We hypothesised that LKB1 and the mTOR pathway are implicated in the pathogenesis of OS.

We investigated the activation of the mTOR pathway in a cohort of 150 OS by immunohistochemistry (IHC) on tissue microarrays. Based on p-mTOR and/or p-p70S6K expression, there is evidence that 77% are potentially responsive to mTOR inhibitors, such as Rapamycin or its analogues. As 97 % of the tumours are also immunoreactive to p-AKT, patients may also benefit from combined therapy against m-TOR and AKT. No correlation was found between the pathway activation and patient response to neo-adjuvant chemotherapy. LKB1 protein expression was present but weak in 90% of the cases by IHC. No cases showed copy number loss for LKB1 gene by fluorescent in-situ hybridisation. The presence of somatic mutations or promoter methylation of this gene is under further investigation.

P9

Expression of DPPA3 (STELLA), EDR1 (PHC1), GDF3 and NANOG, putative stem cell genes on Chromosome 12, in breast carcinoma: a preliminary study

C Ruangpratheep[1], J Shaw[1], RA Walker[1]
[1]Department of Cancer Studies and Molecular Medicine, University of Leicester

According to the concept of breast stem cells and carcinogenesis, stem cell associated genes on chromosome 12 could be reactivated in the development and progression of breast cancer. The aim of this study was to investigate the expression of putative stem cell genes on chromosome 12 in the breast. Expression of DPPA3 (STELLA), EDR1 (PHC1), GDF3, and NANOG mRNA was evaluated in 7 breast cancer cell lines (BCA CL) (HBL-100, MCF7, MDA-MB-231, MDA-MB-436, MDA-MB-468, T47D, and ZR-75-1), 28 normal breast (NB) tissues from reduction mammoplasty, 38 surrounding normal (SN) tissue from breast carcinoma specimens, 3 ductal carcinoma in situ (DCIS), 52 invasive breast carcinoma (IC), and 1 mixed germ cell tumour cell line (NCCIT) using 40 cycles of TaqMan® quantitative reverse transcriptase polymerase chain reaction (QRT-PCR). DPPA3 was expressed in 1/28 NB, 7/38 SN, 6/7 BCA CL, and 14/52 IC. By comparison with NB at the 95% confidence interval, EDR1 was significantly up-regulated in 2 SN, 2 BCA CL, and 2 IC; NANOG was significantly over-expressed in 11 SN, 4 BCA CL, and 5 IC. The tumours without axillary lymph node metastasis were more likely to have up-regulated NANOG (relative risk = 2.482 and odds ratio = 4.622). GDF3 mRNA was not detected in BCA CL and other tissues. In conclusion, over-expression of NANOG merits further investigation for its role in development and progression of breast carcinomas.

P10

Validation of metasin: a novel real-time PCR assay for metastatic carcinoma

S Al-Ramadhani[1], P Balaraman[1], A Mascall[1], E Arkoumani[1], J McKenzie[1], S Jader[1], S Al-Sam[1], S Jenkins[1], H Bradpiece[1], A Patel[1], M Morgan[1], V Sundaresan[1]
[1]Princess Alexandra Hospital

Introduction and Method : We have validated a rapid intraoperative test for metastatic breast cancer in Sentinel Nodes. This assay utilises three distinct markers: CK-19, MGB and PIP. The assay also incorporates an internal reference gene: PBGD.
We have to-date analysed 13 positive sentinel nodes from a cohort of 130 nodes from 60 patients.
Results : We have validated our data in parallel with those obtained on the Veridex – BLN assay. A high level of concordance was observed; however, 3 of 143 nodes showed discordant results.
In 2 of these, histology was negative whilst the Veridex assay was positive. In the third case, the Veridex – BLN assay was negative, histology was positive (a macrometastasis). Our analysis revealed positive CK-19 and PIP for this sample.
Conclusion : The significance of these observations indicate that all discordant samples should be independently verified using an alternate non-BLN assay. The validation of the novel Roche-based assay are discussed.
The above data was obtained using a hybridisation-based RT-PCR strategy using the LightCycler 2.0 platform. However, the hydrolysis-based probe system potentially provides a more robust and versatile assay enabling incorporation of more probes for multiplex-based assays, to improve the sensitivity. Here we compare our data obtained with hybridisation probes with a hydrolysis-based probe set.

P11

Early rapid diagnosis of metastatic cancer in liver and lung biopsies and malignant fluids using Metasin: a Multiplex-RT PCR-based assay

S Al-Ramadhani[1], P Balaraman[1], A Mascall[1], M Morgan[1], V Sundaresan[1]
[1]Princess Alexandra Hospital

Introduction: A wide variety of tumours present clinically as metastatic disease. Here, we describe preliminary data using a range of different probes in a multiplexed RT-PCR assay (Metasin) to detect metastatic disease in a time frame of 40 to 60 mins.
Methodology: The principle of the test is to demonstrate the expression of an alien (non-native for that tissue) mRNA transcript in biopsies and malignant effusions using RT-PCR. Technological advances in fluorescence probes and their detection imply the reality of multiplexing probes sets.
Results: For our assay, we have identified several probes, some already in use as very robust antibodies eg. TTF1 with very high level of sensitivity and specificity.
Preliminary data indicate the feasibility to detect metastatic lung cancer using TTF1- expression-based reagents in addition to NapsinA, Surfactant protein-B. Other markers evaluated include CGA, NCAM1, Desmocollin3 and ALDH3A1. We have converted these probe sets from hybridisation probes to hydrolysis-based probes for use on the Roche 480 Light-Cycler platform. We present data to demonstrate the specific detection of metastatic breast and lung cancer in liver and lung biopsies in addition to metastatic disease in both pleural effusions and ascites from these sites.
Conclusion: Use of Metasin in parallel with H&E sections will provide important data for pathologists for rapid and early diagnosis of cancer.

P12

Molecular characteristics of small cell lung carcinoma

MF Bari[1], D Zehnder[1], J Moore[1], H Brown[1], **D Snead[2]**
[1]University of Warwick, [2]University Hospitals Coventry and Warwickshire

Lung Cancer is the leading cause of the cancer related deaths in the UK. Small Cell Lung Carcinoma (SCLC) accounts for around 20% of cases, and is the most aggressive form. Considerable research efforts has been invested in understanding the molecular events which characterize the different types of lung cancer, but relatively little data is available on SCLC with most studies relying on cell lines. A method of combining results from the different array platforms used in expression profiling of these tumours, would be useful in identifying biomarkers useful for diagnosis and treatment of these tumours. Following a systematic review we combined the data of different studies by tabulating genes identified in two or more studies, and ranking them by fold change. We then compared the position of genes with the published data on loss or gains of genomic DNA.
159 up and 105 down regulated genes in SCLC were common to 3 or more studies. 29 up regulated genes and 15 down regulated genes were also reported in Adeno (ADC) or Squamous carcinoma (SCC), the remainder were specific for SCLC. These included ASCL1, STMN1, INSM1, DDC, SGNE1, SOX4 (top 6 up) and CAV2, ANK3, TACSTD2, BLVRB, ANXA2, LAMA3 (top 6 down).
This study shows that data from different studies using different platforms can be combined, using fold change of expression, to provide useful data on rarer tumour types. Furthermore we report a list of putative biomarkers likely to be relevant in the treatment or diagnosis of SCLC.

P13

Ret rearrangement is associated with young age at diagnosis not radiation exposure

K Unger[1], T Bogdanova[2], V Pushkariev[2], M Tronko[2], GA Thomas[1]
[1]Imperial College London, [2]Institute of Endocrinology and Metabolism, Kiev

One problem in interpretation of early studies on the molecular biology of papillary thyroid cancer (PTC) associated with radioiodine exposure post Chernobyl was the lack of an age matched cohort as a control. The Chernobyl Tissue Bank (CTB) is a prospective collection of thyroid tumours from the areas of Ukraine and Russia contaminated by fallout from the Chernobyl accident on 26/4/86. RNA from 56 cases of PTC, (34 born before 26/4/86 (exposed) and 22 born after 1/1/87 (non-exposed) – matched on age, residency and gender) was supplied by the CTB. Primers located in exons 2/3 (extracellular: EC) and 12/13 (tyrosine kinase: TK) were used to identify expression of the Ret gene using qRT-PCR. PTC1 and 3 were identified by rearrangement specific qRT-PCR. There was no significant difference in the frequency of Ret rearrangement (15/34 (44%) and 11/22 (50%)) or PTC3 involvement (7/15 and 6/15). 6/15 and 5/11 cases harboured rearrangements other than PTC1 and 3, and 3 cases in the exposed group were PTC1 and 1 case was positive for both rearrangements. One case in the exposed group showed unbalanced expression of the EC domain. The level of expression of Ret was variable, suggesting either subclonal rearrangement within the tumour or differential expression associated with different rearrangements. These results suggest that Ret rearrangement is associated with young age at onset, not radiation exposure.

P14

The role of post operative radiotherapy in the management of parotid pleomorphic salivary adenoms: is there any benefit?

M Atwan[1], L Cooper[1], AG Robertson[1]
[1]Glasgow Royal Infirmary

Introduction: Pleomorphic Salivary Adenoma is the most common tumour of the Parotid Gland. Currently no guidelines exist on the management of these tumours. Patients are either treated by surgery alone, or adjuvant radiotherapy is added. Our objective is to evaluate the role of adjuvant radiotherapy.
Methods: A retrospective analysis of all patients who underwent PSA excision in Strathclyde from 1981–2008. We reviewed adherence to the facial nerve, excision margins ,intact or breeched capsule, referral for radiotherapy and its' indications, recurrence rates and iatrogenic complications.
Results: 201 patients were treated for PSA. 167 by surgery alone and 34 by surgery plus radiotherapy. Notes were retrievable in all who had radiotherapy and in only 58 patients who had surgery alone. 1 out of 58 patients suffered a recurrence, while 1 of the 34 patients to receive radiotherapy suffered a recurrence. 100% who received radiotherapy suffered shot term complications and 13/34 (38%) patients suffered long term complications relating to radiotherapy. 12/34 (32%) suffered long term complications due to surgery. 15/58 (25%) who were treated by surgery alone suffered long term complications. In total 20/34 (59%) patients who underwent radiotherapy suffered long term complications compared to 15/58 (26%) from surgery alone group.
Conclusions: There was no significant difference in recurrence.Short and long term complications were significantly higher in the radiotherapy group. 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.

P15

Distribution of serotonin receptors 5-HT$_{5A}$ during neurulation

E Mora-Loyola[1], **P Moreno-Layseca**[1], J Escobar-Cabrera[1], LC Berumen[1], A Rodríguez-Torres[1], G García-Alcocer[1]
[1]Faculty of Chemistry, Autonomous University of Querétaro, Querétaro 76010, México

Neural tube defects are the second most frequent type of congenital malformations which occur during the neurulation, a crucial process in the development of the nervous system. Changes during neurulation could be associated with chemical substances. One of the substances associated with the formation and development of the central nervous system is serotonin, which is known to play a role in brain development prior to the time it assumes its role as a neurotransmitter in the mature brain. Serotonin effects are mediated by the activation of its receptors, organized in several subtypes, like the 5-HT$_{2B}$ and 5-HT$_{5A}$. The 5-HT$_{2B}$ subtype is reported to have trophic functions, while the 5-HT$_{5A}$ receptor's function remains unknown. Studies focused on researching the potential role of the 5-HT$_{5A}$ receptors in the neurulation could help to understand the causes of neural tube defects. In this work the distribution of serotonin receptors 5-HT$_{5A}$ in chicken embryos neurulation was evaluated using immunohistochemistry. The results showed a differential expression of the 5-HT$_{5A}$ serotonin receptors in somites, caudal region and encephalon in the three stages assayed. The distribution was higher in the early stages and it decreased with the ontogeny of the embryo, suggesting a role of these receptors in the development of the central nervous system.

P16

Utility of sarcoma-specific fusion gene analysis in paraffin-embedded material for routine diagnosis at a specialist centre

K Thway[1-2], S Rockcliffe[1], D Gonzalez[2], GJ Swansbury[3], T Min[3], L Thompson[2], C Fisher[1]
[1]Department of Histopathology, Royal Marsden Hospital, London, UK, [2]Institute of Cancer Research, Sutton, Surrey, UK, [3]Department of Haemato-Oncology, Royal Marsden Hospital, London, UK.

Soft tissue sarcomas are rare and correct histopathological diagnosis is essential for treatment, but there is frequent morphological and immunohistochemical overlap between subtypes, and with other lesions. Many sarcomas have specific genetic aberrations detectable by molecular methods, which can be applied to paraffin-embedded material, especially as fresh/frozen material is often unavailable. We assessed the effectiveness and limitations of molecular genetics as part of a routine diagnostic service. A retrospective audit was performed of consecutive patients over 14 months, evaluating diagnostic usefulness of fluorescence in-situ hybridisation (FISH) and real-time polymerase chain reaction (RT-PCR) as part of the standard repertoire of molecular tests for sarcomas. Results from each method were compared with histology. 158 patients had genetic tests (total 194 RT-PCR, 174 FISH). Synovial sarcoma, Ewing sarcoma and low-grade fibromyxoid sarcoma were the most commonly tested tumours. Myxoid liposarcoma was the most sensitively tested, whereas alveolar rhabdomyosarcoma yielded the most frequently concordant PCR and FISH results. FISH was more sensitive than PCR in sarcoma detection, with a better success rate, although PCR was useful for determining specific fusions. FISH and RT-PCR on paraffin-embedded tissue are effective ancillary tools for sarcoma diagnosis. FISH has higher sensitivity for detecting tumours, and a higher success rate than RT-PCR, which is dependent on adequate RNA extraction, particularly from referral blocks in which methods of tissue fixation and processing vary. Their continued use as complementary methods is optimal for maximising the detection rate of sarcomas with translocations.

P17

Dedifferentiated liposarcoma with meningothelial-like whorls, metaplastic bone formation and CDK4, MDM2 and p16 expression: a morphological and immunohistochemical study

K Thway[1], C Fisher[1]
[1]*Department of Histopathology, Royal Marsden Hospital, London, UK.*

We analysed four cases of dedifferentiated liposarcoma with meningothelial-like whorls and metaplastic bone formation (DLWMB), assessing morphology and immunohistochemical expression of a panel of antigens, including CDK4, MDM2 and p16 proteins, to characterise further these rare morphological features. The specimens were from the retroperitoneum or pelvis, and all four comprised exclusively or predominantly dedifferentiated liposarcoma. All cases showed prominent metaplastic bone, while three additionally showed cartilage. Furthermore, one of the cases showed heterologous osteosarcoma, never previously described in the context of DLWMB. The whorls were composed of concentric distributions of morphologically distinct spindle or epithelioid cells, with one case showing both cell types. All cases expressed smooth muscle actin, two diffusely, two focally, while three cases showed claudin-1 positivity. In all cases the whorls expressed at least two of CDK4, MDM2 and p16. While the differentiation of the whorls is still not fully elucidated, the presence of two morphological subsets and immunohistochemical expression towards two distinct lines of differentiation suggests that the whorls represent heterogeneous structures of different lineages, with possible myopericytic and perineurial lines of differentiation. The CDK4, MDM2 and p16 expression in the whorls suggests that they share a similar genetic background to well-differentiated and dedifferentiated liposarcoma, and that additional genetic events are causal to their distinct morphology.

P18

Beta catenin expression in paediatric fibromatoses: a study of 100 fibromatous and myofibromatous lesions

K Thway[1], S Gibson[2], A Ramsay[3], NJ Sebire[2]
[1]*Department of Histopathology, Royal Marsden Hospital, London UK,*
[2]*Department of Paediatric Histopathology, Great Ormond Street Hospital, London UK,* [3]*Department of Histopathology, University College Hospital, London UK*

Aims: Nuclear immunoreactivity for beta-catenin is a useful adjunct for diagnosis of adult desmoid-type fibromatoses, many of which exhibit mutations within the APC/beta-catenin (Wnt) pathway. Paediatric fibromatoses represent a heterogeneous group of lesions which are diagnostically challenging, especially on biopsy. We studied beta-catenin expression in a variety of paediatric fibroblastic and myofibroblastic lesions.
Method and Results: Immunohistochemical nuclear expression of beta-catenin was assessed in 100 tumours. High-level expression of beta-catenin was found in 42% of usual-type or deep fibromatoses (21/50). Such expression was not seen in any of the other lesions, including: fibrous hamartoma of infancy (0/18), juvenile hyaline fibromatosis (0/7), infantile digital fibromatosis (0/6), myofibromatosis (0/5), lipofibromatosis (0/4), calcifying aponeurotic fibroma (0/3), palmar-plantar fibromatosis (0/2), fibromatosis colli (0/1) or torticollis (0/1).
Conclusion: High-level beta-catenin staining is seen in deep 'adult-type' fibromatoses occurring in children, although to a lesser frequency than in adult fibromatoses. This suggests that a subset of deep fibromatoses in childhood shares similar mechanisms of tumorigenesis to those in adults. beta-catenin is not expressed in other common paediatric fibroblastic and myofibroblastic lesions and the Wnt pathway does not appear to play a role in their pathogenesis.

P19

Claudin-1 is expressed in perineurioma-like low grade fibromyxoid sarcoma

K Thway[1], C Fisher[1], M Debiec-Rychter[2], E Calonje[3]
[1]*Department of Histopathology, Royal Marsden Hospital, London, UK*
[2]*Centre for Human Genetics, Catholic University of Leuven, Belgium*
[3]*St John's Institute of Dermatology, St Thomas' Hospital, London, UK*

Low-grade fibromyxoid sarcoma is a soft tissue sarcoma with recurrent and low metastatic potential, which has characteristic FUS-CREB3L2 or FUS-CREB3L1 fusions. Perineurioma is a peripheral nerve sheath neoplasm, which is usually benign. Low-grade fibromyxoid sarcoma and perineurioma can appear morphologically similar, particularly in small biopsy specimens, and distinction between the 2 entities is important for appropriate treatment. Low-grade fibromyxoid sarcoma is negative for most immunohistochemical markers, whereas perineuriomas stain variably for epithelial membrane antigen, CD34 and claudin-1, a tight-junction associated protein. We studied 15 cases of genetically proven low-grade fibromyxoid sarcoma that at least focally resembled perineurioma, with antibodies to claudin-1 and epithelial membrane antigen. Of these, 11 showed positivity for epithelial membrane antigen and all 15 were positive for claudin-1; in all cases, expression of claudin-1 was equal to or greater than the corresponding epithelial membrane antigen expression. This study emphasizes that claudin-1 is significantly expressed in low-grade fibromyxoid sarcomas. This has implications toward the accurate diagnosis of both tumors, and, as positivity for claudin-1 in low-grade fibromyxoid sarcoma is not previously documented, suggests that there might be underdiagnosis of low-grade fibromyxoid sarcoma. Although positivity for claudin-1 remains useful as an adjunct marker for perineurioma, it should be taken in context with clinical findings, morphology, and the additional immunoprofile.

P20

Apparent vascular invasion in melanocytic naevi

PJ Tadrous[1]
[1]*Cellular Pathology Dept, Northwick Park Hospital, London*

Background: When is vascular invasion an acceptable finding in benign melanocytic lesions of the skin? Historically (in 1935) there have been rare reports of 'benign metastasising naevi' to lymph nodes which included intra-sinus involvement of the node suggesting an intravascular route – but this was in an era before immunocytochemistry.
Case Report: A clinically, histologically and immunocytochemically benign intradermal melanocytic naevus with a neural component is presented. This showed apparently separate intravascular melanocytic nests which further investigation demonstrated were subendothelial protrusions and not 'true' vascular invasion.
Discussion: While there have been reports in the literature of vascular invasion or protrusion of neural tissue in neuronaevi and neurofibromata, no report has been found describing this phenomenon with regards to typical melanocytic naevus cells. The implications of this finding are discussed and compared to current concepts of vascular invasion in other situations (i.e. follicular neoplasms in the thyroid). As a result, guidelines for interpreting its significance in melanocytic naevi are suggested. This phenomenon may cause particular concern if it arises in naevi showing cytological and / or architectural atypia and awareness of its existence should help avoid over-diagnosis of malignant melanoma.

P21

Ductal carcinoma arising from a syringocystadenoma papilliferum in a Naevus Sebaceous of Jadassohn: the use of immunohistochemical markers to recognise invasion

LJ Smith[1], EA Husain[1]
[1]*Aberdeen Royal Infirmary, Aberdeen*

Naevus sebaceous of Jadasshon (NS) is a hamartomatous cutaneous lesion involving the epidermis, hair follicles, sebaceous glands and apocrine glands. Many types of cutaneous neoplasms, the majority of which are benign, have been associated with NS. Association with a malignant neoplasm is exceedingly rare, the most commonly reported malignant neoplasm being basal cell carcinoma. We report a case of ductal carcinoma arising subjacent to a syringocystadenoma papilliferum (SCP) in a naevus sebaceous of Jadassohn in a 50 years female patient. Clinically, the lesion presented as a raised pink fleshy lesion of scalp that had been present since birth, but had become enlarged and ulcerated over the last 8-12 months. Histological examination revealed a syringocystadenoma papilliferum arising within a naevus sebaceous of Jadassohn. Just in the dermis subjacent to the SCP, small ductal structures separated by a dense desmoplastic stroma with retained outer myoepithelial layer (positive staining for CK14, p63, SMA and S100) reminiscent of sclerosing adenosis of the breast were seen. At the periphery of this area, some larger ducts with a cribriform pattern and punched out lumina similar to breast ductal carcinoma in situ (DCIS) were also present. These DCIS like ducts retained an outer myoepithelial layer. Admixed with the sclerosing adenosis-like areas there were focally infiltrative tubular glands lacking an outer myoepithelial layer with the appearance of invasive ductal carcinoma.
 This is an exceedingly rare case reflecting the marked similarities between cutaneous ductal carcinoma and breast carcinoma. Immunohistochemistry proved very helpful in identifying the invasive component.

P22

Audit of positive margin rates and accuracy of clinical diagnosis for cutaneous squamous cell carcinomas in the Maidstone and Tunbridge Wells (MTW) NHS Trust

M Varia[1], L Munthali[1]
[1]*Maidstone and Tunbridge Wells NHS Trust*

Aim: To improve the rate of complete excision rates of SCCs received in the MTW trust.
Standards: The rate of incomplete excision for SCCs has a national range of 0% to 10.1% according to the National Institute of Clinical Excellence (NICE). Method: Histopathology reports were obtained for cutaneous SCCs submitted between 01/01/07 to 31/12/07 at the MTW NHS Trust. Data were analysed according to completeness of excision, clinician's speciality, accuracy of clinical diagnosis and site of lesion.
Results: 94 cutaneous lesions with a confirmed diagnosis of SCC were received during the 12 month period. 26.6% were reported as incompletely excised. Incomplete excision rates were 25% for General practitioners (GPs), 23.1% for dermatologists and 31.0% for the 'Hospital Other' category. GPs had the highest complete excision rate (73.3%). 31.0% of SCCs were accurately clinically diagnosed. Dermatologists accurately clinically diagnosed 61.5% of SCCs, followed by Hospital Others (41.4%) and GPs (17.3%).Lesions from the head and neck revealed the highest incomplete excision rate (48.0%).
Conclusion: Incomplete excision rates for SCC in the MTW NHS Trust (26.6%) exceed the national range of 0% to10.1%. Improvements can be made by supporting GPs in improving their knowledge of recognising SCCs clinically and by referring patients with lesions suspicious of malignancy to a team specialising in skin cancer.

P23

The connective tissue microenvironment of the muscularis propria of the human gastrointestinal tract: specificity and selectivity of collagen subtype distribution

J Martin[1], A Ali[1], T Hannan[1], CA Evagora[1], CH Knowles[1]
[1]*Barts and The London School of Medicine and Dentistry*

Fibrosis is a common feature of gastrointestinal disease, including myopathy and may result from alteration of smooth muscle phenotype from contractile to synthetic. Little is known, however, of the normal smooth muscle tissue microenvironment in the normal bowel in humans.
We employed a screening panel including immunochemistry for collagens I, III and IV, tenascin, fibronectin, fibrillin-1, smooth muscle actin, vimentin, desmin, and methods including haematoxylin and elastic Van Gieson, Congo red and periodic acid Schiff. Five disease free human ileal and five human colonic specimens were examined, and the location, distribution and semiquantitatively assessed intensity of staining determined by two observers. All immunostaining included positive and negative controls and sections stained in duplicate for reproducibility.
The ileum and colon showed similar results. Smooth muscle actin and desmin staining was confined to the cytoplasm of smooth muscle cells. Highly specific surface staining of smooth muscle cells was seen with collagen IV, without other staining. This pattern was not evident with other markers. Fibrillin-1 staining, whilst staining fibroblasts, demonstrated focal intensities around smooth muscle cells, with lower level staining between cells. Collagen III was present in abundance and moderate fibronectin staining was seen between cells. Collagen I staining was largely confined to the submucosal regions. Elastic tissue is present in septa of the muscularis propria and the subluminal region of this layer.
This defines a clear model of the tissue microenvironment of smooth muscle of the normal human muscularis propria which is structurally complex and specific at a molecular level.

P24

Audit of lymph node yield and prognostic factors in colorectal cancer pathology reporting after the initiation of NICE Guidelines (2004) in a District General Hospital

E Knight[1], **H Abdelsalam[1]**
[1]*Royal Shrewsbury Hospital NHS Trust*

Objective: To audit pathology reporting standards in a District General Hospital in accordance with National Institute of Health and Clinical Excellence (NICE) and Royal College of Pathologists guidelines.
Methods: A prospectively maintained registry for surgical colorectal cancer resection was interrogated to compare lymph node yield, extramural vascular invasion, tumour perforation and margin involvement in patients treated before (2002-2003) and after (2007-2008) the implementation of NICE guidelines. Categorical data were compared using Chi-square analysis.
Results: 426 patients were identified in 2002-2003 and 430 in 2007-2008 with a median lymph node retrieval of 10 and 14 respectively (p=0.0001). Although national standards were achieved in 2007-2008 sub-classification of these patients by Dukes staging showed 31 Dukes A and 67 Dukes B patients (23% of the study population) had less than 12 lymph nodes analysed.
Identification of extramural vascular invasion increased from 20% to 33% in 2007-2008. This trend was also observed for the reporting of tumour perforation with a 10% increase for colon cancer and a 3% increase for rectal cancer between the two time periods.
Conclusion: Departmental pathology reporting of colorectal cancer improved significantly after the initiation of NICE guidelines in 2004. Despite this, a proportion of the study population had less than 12 lymph nodes retrieved increasing the risk of disease under-staging. To maximise lymph node yield we would recommend fat clearance techniques in specimens where less than 12 lymph nodes have been detected at initial examination.

P25

GEWF solution increases the yield of lymph nodes from colorectal cancer specimens

I Chandler[1], S Glass[1]
[1]*Royal Devon and Exeter Hospital Pathology Department*

Purpose: Accurate staging and treatment of colorectal cancer depends on dissection of sufficient numbers of lymph nodes from excised specimens. The use of glacial acetic acid, ethanol, distilled water and formaldehyde (GEWF) solution to improve lymph node yield over traditional formaldehyde was investigated.

Method: Over six months in 2009, a random subset of colorectal cancer resection specimens was fixed in GEWF rather than formaldehyde. The number of lymph nodes dissected from the specimens was compared to see if there was any significant difference between the fixatives.

Results: Colon cancer specimens fixed in formaldehyde (n = 23) yielded a mean of 14.22 lymph nodes compared to those fixed in GEWF (n = 27), that yielded a mean of 19.89 nodes (p = 0.004).

Rectal cancers fixed in formaldehyde (n = 29) yielded a mean of 11.07 lymph nodes compared with those fixed in GEWF (n = 17), that yielded a mean of 17.82 nodes (p = 0.0016).

Using GEWF solution resulted in no difference between the numbers of tumour positive lymph nodes found in colon (p = 0.7) and rectal (p = 0.57) cancer specimens.

Conclusion: The total number of lymph nodes found in colon and rectal cancer specimens is higher if they are fixed in GEWF solution, but there is no difference in the number of tumour positive nodes found.

P26

An audit of pouch biopsy reporting

DJ Royston[1,2], BF Warren[1]
[1]*Department of Cellular Pathology, John Radcliffe Hospital, Oxford*
[2]*Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford*

Pouchitis is an idiopathic inflammatory condition affecting up to 50% of ulcerative colitis (UC) pouch patients. The diagnosis is made on combined clinical, endoscopic and histological features. The St Marks histological scoring system is used routinely. Pouch biopsies are given acute inflammatory scores of up to 6 and chronic inflammatory scores of up to 6. An acute score of 4 or more (neutrophils and ulceration) correlates with clinical and endoscopic features of pouchitis.

To study the consistent application of these histological criteria, 100 pouch biopsy reports were reviewed. In 58 cases (58%) a description of the endoscopic findings was provided. In 29 cases (29%) a clinical history was provided. In 4 cases (4%) biopsies from the pre-pouch ileum, pouch and columnar cuff were taken. Biopsies from the pouch alone were received in 70 cases (70%). Histological findings consistent with pouchitis were reported in 20 cases (20%), of which 4 cases (25%) were not scored. In 6 cases (30%) with histology reports of 'pouchitis' acute scores of <4 were misleading.

The St Marks score for pouchitis was used in most reports. However, there is a need for more widespread understanding of the criteria for pouchitis. Histological scoring must be applied accurately and consistently to contribute to the diagnosis of pouchitis.

P27

Helicobacter Pylori: development and characterisation of a novel mouse monoclonal antibody

JN Walker[1], NH Piggott[1], M Hall[1], MJS Lee[1], S Cowell[1], T Murphy[1], PW Scorer[1], C Dillon[1], M Rees[1], M Pinkney[1], GG McIntosh[1], ID Milton[1], A Riba[2], T Ingeneri[2], P Seigler[2], L Bird[2].
[1]*Leica Biosystems Newcastle Ltd,* [2]*Plus Diagnostics USA*

A marker that would specifically identify Helicobacter pylori (H. pylori) and not cross-react with the related and morphologically similar Campylobacter species, or indeed other species of Helicobacter, would be of value both in clinical practice and for use in research.

The genetic code corresponding to a truncated region of HopE has been cloned and expressed in Escherichia coli bacteria and the purified recombinant protein used as an immunogen for the generation of a monoclonal antibody. The HopE immunogen was designed to ensure that it shared no homology with any C. jejuni proteins. In addition, homology with proteins of Helicobacter species other than H. pylori was seen to be minimal.

Hybridoma clones secreting specific H. pylori monoclonal antibody were produced by standard techniques. In western blot analysis clone ULC3R recognised a single band in H. pylori cell lysate of 29-30kDa corresponding to the molecular weight of the HopE protein. In immunocytochemical analysis, polyclonal antibody NCL-HPp crossreacted with C. jejuni in a FFPE cell block of C. jejuni bacteria. In contrast, clone ULC3R, displayed no staining of C. jejuni.

A comparative immunohistochemical study was performed with clone ULC3R and NCL-HPp on biopsies from 300 cases of H. pylori–associated gastritis using the Bond automated system. Clone ULC3R displayed more defined morphology of H. pylori bacteria and cleaner staining than NCL-HPp. Sensitivity and specificity demonstrated by the new H. pylori monoclonal antibody, clone ULC3R, indicated that it will be useful for specific detection of H. pylori in histological sections

P28

18cm Mullerian cyst attached to caecum in a postmenopausal woman

K Dasgupta[1], JN Bulmer[1], B Haugk[1]
[1]*Royal Victoria Infirmary*

We describe a rare case of a Mullerian cyst attached to the caecal wall in a 70 year old lady presenting with long standing dysuria and low back pain. Imaging revealed a 20 cm fluid filled cyst extending from the right hypochondrium to iliac fossa close to the ascending colon and separate from the kidney. Tumour markers and hydatid serology were negative. Laparoscopy suggested a mesenteric cyst attached to the ascending colon. Following aspiration fluid reaccumulated and the cyst was removed by a right hemicolectomy.

Macroscopically a thin walled (<1mm) unilocular 18cm cyst was attached to the anterolateral caecal wall and drained clear serous fluid. No solid nodules were seen.

Microscopically the fibrocollagenous cyst wall was lined by single layered ciliated low cuboidal epithelium without stratification or atypia. The epithelium expressed CK7, oestrogen and progestrerone receptors and CA125 showed apical positivity. CK20, TTF1 were negative and there was no CD10 positive stroma.

Upper abdominal Mullerian cysts are rare and enter into the differential diagnosis of retroperitoneal cysts including reduplication cyst, cystic mesothelioma and lymphangioma. Malignancy in upper abdominal Mullerian cysts has not been described. Post menopausal presentation and caecal attachment of such cyst is a rare occurence.

P29

Taurochenodeoxycholic acid activates NF-KB in oesophageal squamous cells without accompanying loss in cell viability

Z Nichols[1], NH Green[2], BM Corfe[3], S Macneil[2], **JP Bury[1]**
[1]*Academic Unit Pathology, University of Sheffield Medical School*
[2]*Kroto Research Institute, University of Sheffield,* [3]*Human Nutrition Unit, University of Sheffield Medical School*

Exposure of eosophageal squamous mucosa to bile salts in duodenogastric refluxate is implicated in the pathogenesis of Barrett's oesophagus. Previous studies have suggested that bile salts activate NF-KB which in turns leads to the transcriptional activation of CDX2. A variety of different bile salts are found in refluxate and it is possible that geographic variations and temporal trends in the incidence of Barrett's oesophagus may relate to dietary-related variations in the concentrations of bile salts with differential toxicities. There has been limited systematic study of the effects on squamous cells of individual bile salts - which are at least in part determined by their differing pKa values. We studied the impact of pulsatile exposure of seven bile salts on immortalised oesophageal squamous (HET1A) cells at neutral pH and pH 4. Bile salts were studied singly and in a physiologically representative mix. Cell viability was measured by MTT-ESTA assay and activation of NF-KB was assessed using immunocytochemistry and high-content image analysis. NF-KB activation was seen for many of the individual bile salts but in most cases this was accompanied by a loss in cell viability. However, the taurine conjugated bile salt taurochenodeoxycholic acid at pH4 led to the activation of NF-KB without accompanying loss of cell viablity. This specific effects of TCDC is particularly interesting since an increased level of taurine conjugated bile-salts is associated a diet high in dietary animal-fat which may correlate with the epidemiology of Barrett's oesophagus.

P30

Advances in multiresolution serial section 3D reconstruction: applications to liver microarchitecutre

PJ Tadrous[1], A Hall[2], AP Dhillon[2]
[1]*Cellular Pathology Dept, Northwick Park Hospital, London,*
[2]*Histopathology Dept, Royal Free Hospital, London*

Background: Traditional methods of 3D reconstruction (3DR) from serial tissue sections are difficult and rely on interpretative tracing of objects of interest. Knowledge of human liver microanatomy remains incompletely understood and is important to understanding conditions such as hepatocellular carcinoma, cirrhosis and regeneration. Here we present results of the latest developments in 3DR as applied to healthy liver tissue with the aim of investigating microvascular relationships.
Methods: 134 H&E-stained serial sections of human liver were subjected to automated registration and 3D modelling at x20 and x40 magnification. A second set of 30 serial sections were stained using a reticulin haematoxylin method (which we designed to highlight the sinusoidal network with a colour contrast optimal for detailed 3DR) and imaged at higher magnification (x600). A novel 'virtual dye injection' technique is introduced that highlights selective aspects of the vascular tree without resorting to manual tracing. The 3D models were made using FATCAM (the Flexible Assignment of Transparency and Colour-based Alpha Mapping protocol) and examined interactively using VRML (the Virtual-Reality Modelling Language).
Results and Discussion: Our models of the portal, systemic and sinusoid systems are presented in 3D. In addition to illustrating some previously published theories, these models also suggest that the earliest sign of central venule formation is localised sinusoidal dilatation (or hyperanastomosis). We also demonstrate that the systemic venous system receives tributaries in both a hierarchical and non-hierarchical manner. These models are unique in being derived from the tissue components themselves and not user-traced objects.

P31

Autofluorescence Spectroscopy – developing a tool for the assessment of steatohepatitis in the liver

AP Levene[1], QM Anstee[2], H Kudo[1], S Ladva[1], N Thanh Hoa[3], V Sauvage[3], D Concas[2], MR Thursz[2], DS Elson[3], RD Goldin[1]
[1]*Department of Histopathology, Imperial College Faculty of Medicine at St Mary's, London, UK,* [2]*Department of Gastroenterology & Hepatology, Imperial College Faculty of Medicine at St Mary's, London, UK,* [3]*Department of Biosurgery & Surgical Technology and Institute of Biomedical Engineering, Imperial College, London, UK*

Background & Aims: Liver biopsy is the gold standard to differentiate non alcoholic steatohepatitis (NASH) from simple steatosis in cases of non alcoholic fatty liver disease (NAFLD). However, this is an invasive procedure and has associated morbidity. Autofluorescence spectroscopy is a powerful approach for real-time, minimally invasive characterisation of tissue oxidative stress based on the levels of endogenous fluorophores in the liver. We sought to determine whether autofluorescence could be used to distinguish steatosis from NASH in a mouse model of NAFLD.
Methods: Three groups of mice were compared: (A) the MCD model of fibrosing steatohepatitis; (B) a model of mild steatosis (Db/Db mice); (C) normal liver. Fluorescence spectra were recorded from the surface of the explanted liver with a probe comprised of one excitation and six emission optical fibres (core diameter 200μm).
Results: At 375nm excitation, the mean 465nm:530nm fluorescence ratio was significantly lower in steatohepatitis 1.8 ± 0.02 (SEM) than in steatosis 2.19 ± 0.05 or normal tissue 2.08 ± 0.03, ANOVA $p<0.001$. These observations mirrored the changes in TBARS, a measure of lipid peroxidation, $1.79\pm0.46\mu m/100mg$ tissue, 0.28 ± 0.02 and 0.26 ± 0.02, ANOVA $p=0.007$. This correlated with both biochemical and histological measures of inflammation.
Conclusions: Autofluorescence is an effective tool to accurately differentiate steatosis from steatohepatitis in mouse models of NAFLD. Work is in progress to assess this tool in the clinical setting.

P32

Lymphangiogenesis is a feature of progressive chronic liver injury

JC Hutchinson[1], X Xu[1], H Robertson[1], AD Burt[1]
[1]*Institute of Cellular Medicine, Newcastle University*

Neovascularisation is a key component of repair processes and may persist in fibrosis. Proliferation of vascular structures has been documented in progressive liver disease and is accompanied by capillarisation of sinusoidal endothelium. The present study tests the hypothesis that lymphangiogenesis also occurs in liver fibrosis. The distribution and density of intrahepatic lymphatics were assessed in 5 micron sections of 'normal' control liver, acute liver injury (paracetamol overdose), cirrhosis, acute cellular allograft rejection and chronic allograft rejection using immunohistochemistry with D2-40 antibody which detects podoplanin, a marker of lymphatic endothelium. Vascular endothelium (including capillarisation) was detected using anti-CD34. The size and density of vessels were measured using an angiogenesis software module on an Aperio scanner.
Lymphatics were seen in portal tracts of normal liver and in Glisson's capsule. Increased numbers of D2-40 stained lymphatics were seen in septa of cirrhotic livers (p=0.003 cf. normal) and beneath the capsule in chronic injury but were not observed within the areas of capillarisation. The density of lymphatics increased progressively with increasing stage in a subset of primary biliary cirrhosis biopsies. No such increase was seen in acute injury or acute rejection but there was a trend towards increased density in chronic rejection.
We conclude that lymphatic remodelling and lymphangiogenesis occurs as part of the response to chronic liver injury. This may have implications for trafficking of immune cells in the damaged liver and for the development of ascites in cirrhosis.

P33

Percutaneous medical liver core biopsies: correlation between tissue length and the number of portal tracts

J Chan[1], **Y Alwahab**[1], C Tilley[1], N Carr[1]
[1]Department of Histopathology, Southampton General Hospital

Objective: Liver biopsy is an important tool in clinical practice for the diagnosis and assessment of liver disease. However, adequate tissue yield is required for accurate histological evaluation and the Royal College of Pathologist tissue pathway advise a length of at least 10mm and a minimum of 6 portal tracts. We aimed to establish the relationship between biopsy length and the number of total portal tracts.

Methods: We reviewed 163 16G percutaneous medical liver biopsies taken at a tertiary referral centre over a 14 month period. Haematoxylin and eosin sections were analysed using imaging software and portal tracts were counted.

Results: Liver biopsy length correlates with total portal tracts. The median biopsy length was 13.3mm with a median of 6 portal tracts. 88% of the biopsies measured at least 10mm but only 52% contained a minimum of 6 portal tracts. 95% of biopsies measuring at least 15mm contained 6 portal tracts or more with a significant difference between total portal tracts when compared with biopsies measuring less than 15mm (P<0.0001).

Conclusion: Frequently liver biopsies in this series did not meet the recommendations as set by the Royal College of Pathologists. To facilitate adequate histological assessment by examining at least 6 portal tracts, we suggest that 16G biopsies should be at least 15mm long.

P34

Case report: adult mixed malignant tumour of liver with review of literature

A Kempalingaiah[1], TV Luong[1]
[1]Dept of Histopathology, Royal Free Hospital

We present a case of synchronous hepatocellular carcinoma and mixed epithelial and mesenchymal malignant tumour in a 69 year old male patient. He presented with non alcoholic steatohepatitis and hepatitis B associated cirrhosis. The imaging showed features of cirrhosis with multifocal hepatocellular carcinoma. He underwent an orthotopic liver transplant. The explant liver contained two discrete tumours in a cirrhotic background. Histologically, one of the tumours was a well to moderately differentiated hepatocellular carcinoma whilst the other was a mixed epithelial and mesenchymal malignant neoplasia, which presented difficulty in the differential diagnosis between carcinosarcoma and mixed hepatoblastoma. The patient is well 9 months after surgery.

P35

Does human papilloma virus play a role in the development of bladder squamous cell carcinoma?

R Vaziri[1], HI Hassan[2], E Husain[3], **SI Baithun**[4]
[1]Queen's Hospital, BHR Hospitals NHS Trust, [2]Assiut University, Assiut, Egypt, [3]Aberdeen Royal Infirmary, Aberdeen, UK, [4]Barts and The London, Queen Mary University of Medicine and Dentistry

Aim: To investigate the role of human papilloma virus (HPV) in the development of bladder squamous cell carcinoma (SCC).
Methods: paraffin embedded tissue blocks of bladder SCC from 36 British patients and 31 cases from Egypt (total number of cases = 67) were tested for the presence of HPV DNA by a consensus GP5+/GP6+ primer mediated polymerase chain reaction (PCR) technique. For this study we used the conventional agarose gel analysis. 10 control cases with their corresponding fresh frozen tissue were also tested to exclude false negative results.

Results: HPV DNA was detected in 35/67 cases (52.2%), comprising 20/36 (55.5%) of British and 15/31 (48.3%) of Egyptian cases. For the Egyptian cases correlation between HPV status and Schistosoma infection did not show any significant result. There was no significant correlation between HPV status, age and sex.

Conclusion: We previously reported that HPV DNA was not detected in transitional cell carcinoma of the bladder. In this study, the presence of HPV in more than 50% of the cases could suggest a potential contributing aetiology in the development of bladder squamous cell carcinoma.

P36

An audit of renal cancer reporting

P Mairembam[1], A Freeman[1]
[1]University College Hospital, London

Aim: To examine the accuracy and completeness of the pathology report and minimum dataset form in the reporting of renal tumours with regard to categories in minimum dataset. To assess the turnaround time for reporting of renal tumours.

Audit Standards: Royal College of Pathology Dataset for adult renal parenchymal cancer, Nov2006.

Materials and methods: Retrospective data collection for Audit period - August 2006 to August 2007. Data sources – Copath computer system, Pathology Department, UCLH. The histology reports and dataset forms were evaluated for the core data items (laterality, location, adrenal, size, renal vein invasion, tumour type, grade, necrosis, lymphovascular invasion, margins, stage and type of operation).

Results and conclusion: No of cases – 34. Majority of core items are recorded in over 85% of the pathology reports and 88% of the dataset forms. Renal vein invasion grossly and necrosis microscopically is mentioned if present but its absence is not documented in the majority of cases. The absence of a specific category in the pathology report has sometimes been presumed as 'negative' for the dataset rather than being left blank. There was no major discrepancy between the reports and the dataset forms. Turnaround time: Range between 2 to 16 days. Two cases exceeding two weeks needed a second opinion and electron microscopy.

Recommendations: The pathology report should include specific mention of presence / absence of renal vein invasion and necrosis. Absence of a specific category in the pathology report should be left as blank on the dataset rather than being presumed negative.

P37

Analysis of hepsin protein expression in human prostate cancer

M Ho-Kan[1], K Chaudhary[1]
[1]*Barnet and Chase Farm Hospitals NHS Trust*

Hepsin is a cell surface serine protease expressed in several human tissues including prostate. The physiological function of hepsin remains unclear. Expression gene profiling studies suggest hepsin gene to be differentially expressed between benign and malignant prostatic tissue. A correlation with the disease progression has also been reported.

We analysed the immuno expression of hepsin protein in formalin fixed tissues from 59 prostate cancer patients. A polyclonal anti-hepsin antibody utilising a perioxidase anti-peroxidase method of immunhistochemistry was used.

In benign prostate tissue an absent or a weak hepsin expression is seen in the luminal epithelial cells. A significant overexpression is seen in the malignant prostatic glands. There is both cytoplasmic and cell membranous expression in the malignant prostatic acini. No nuclear expression is demonstrated. The degree of immunoexpression varied from weak, moderate to strong in 39%, 54.2% and 6.8% cases of prostate cancers respectively. Most foci of high grade prostatic intraepithelial neoplasia also expressed a higher level of hepsin staining compared to benign glands. A striking and significant finding is that all foci of glandular atrophy are either negative or show a faint hepsin staining. Our results demonstrate hepsin upregulation in human prostate cancer. Evaluation of the cellular localisation and biological functions of Hepsin in prostate cancer may result in its utility as a biomarker and/or therapeutic target.

P38

A comparison of basal cell staining in prostate core biopsies

K Macdougall[1], A El-Sherif[1]
[1]*Department of Histopathology, Royal Victoria Infirmary, Newcastle upon Tyne*

Small foci of atypical glands present a diagnostic problem in prostatic core biopsies. Many immunohistochemical stains are available to aid diagnosis by identifying basal cells.

In our institution the established practice has been to use the high molecular weight cytokeratin 34Beta E12 in difficult cases. Subjective problems with this stain including aberrant non-specific staining of malignant cells have been noted leading to problems with interpretation.

We decided to make a comparison of our current practice (34 Beta E12) against an alternative basal cell marker (CK5/6) to determine the better marker for routine use in the diagnosis of prostatic cancer . We report here on the first stage of our audit.

We performed a prospective audit by applying both 34BetaE12 and CK5/6 to all cases requiring an immunostain over a period of six months. These were examined for the quality of staining, and for the staining of the diagnostically relevant glands.

In 4 cases (5%) 34BetaE12 showed aberrant non-specific staining of the relevant glands. In 3 cases (4%) CK 5/6 showed technical problems which precluded diagnostic assessment. In 1 case CK 5/6 did not show any basal cells, although basal cells were clearly identified on 34BetaE12.

The problems associated with CK5/6 occurred at a similar rate to those experienced with 34BetaE12. We therefore recommend no change in the practice of our institution. Our laboratory will attempt to improve on the technical aspects of CK5/6 which may be of additional use in targeted cases.

P39

Metastatic renal cell carcinoma – an common entity in uncommon locations

C D'Arcy[1], **S Phelan**[1-2]
[1]*St. Vincent's University Hospital, Ireland*, [2]*University College Dublin, Ireland*

Renal cell carcinomas (RCC) are well known for their tendency to metastasise early and to a wide range of sites. Metastatic renal cell carcinomas are frequently encountered in the surgical pathology laboratory both in patients with documented primary RCC and as a first presentation of disease. We report three cases of metastatic RCC in unusual locations: the pancreas, testis and scalp; presenting to our department in quick succession, which illustrate the diverse anatomical patterns of this disease, with a discussion of differential diagnoses.

Renal cell carcinoma remains one of the most lethal urologic malignancies with up to 40% of patients dying of cancer progression. Significant advances have been made in the diagnosis and staging of RCC but approximately one third of patients who undergo surgery for clinically localised RCC will suffer a recurrence. RCC is known to metastasise through haematogenous routes to distant organ sites and via lymphatic channels to regional lymph nodes. The diagnosis of primary or metastatic RCC can be difficult and a variety of histological appearances are described. Relevant clinical information may be commonly omitted and the onus lies on the reporting pathologist to always consider this diagnosis in tumours with a clear cell morphology in any and every site.

P40

Carcinosarcoma of the renal pelvis

T Grigor[1], E McLarty[1], M Powari[1], M Smith[1]
[1]*Derriford Hospital*

We report the rare case of sarcomatoid carcinoma of transistional cell origin arising in the renal pelvis.

The patient was a Caucasian male, aged 80, presented with persistent painless macroscopic haematuria and unexplained microscopic haematuria. He had a past medical history which included low grade Non-Hodgkin's Lymphoma. An uncomplicated laparoscopic left nephroureterectomy was performed.

The cut surface of the kidney demonstrated a pelvi-calyceal tumour measuring 50 x 40 x 40mm.

Histologically the tumour consisted of a high grade tumour, in some areas showing the morphology of transitional cell carcinoma. Diffuse sheets of mononuclear cells with many osteoclast type giant cells were seen along with chondromyxoid stroma. Pancytokeratin stained the areas recognizable as TCC but was negative in poorly differentiated areas.Overall the poorly differentiated areas represented sarcomatous differentiation. The tumour was regarded as carcinosarcoma with probable cartilaginous differentiation. In addition, the renal parenchyma as well as perineal fat showed infiltration by chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL).

Transistional cell carcinoma accounts for most renal pelvis malignancies. Carcinosarcoma of transistional cell origin in the renal pelvis is rare and reported only as case reports in the literature. Foci of osteogenic and chondrogenic differentiation as in this case is rarer still. The reported prognosis of these tumours is poor.

References: Chen KTK et al. Carcinosarcoma of Renal Pelvis. Urology. 1983; Vol 22(4): 429-431

Dimitriou RJ et al/ Carcinosarcoma of the renal pelvis. Urology. 2000; Vol 56(3): 508

P41

Peri-umbilical metstatic nodules in postmenopausal females

MO Samaila[1], GD Waziri[1], AK Koledade[2]
[1]Department of Pathology, Ahmadu Bello University Teaching Hospital
[2]Department of Obstetrics &Gynaecology, Ahmadu Bello University Teaching Hospital

Background: Malignant tumours of the female reproductive organs commonly metastasize to contiguous structures and other sites intra-abdominally, bone and central nervous system. We report three postmenopausal females who presented in our hospital with peri-umbilical nodules diagnosed as metastatic lesions from the endometrium and ovary.

Method: Patients' tissue biopsies were fixed in 10% formalin, processed in wax and stained with Haematoxylin & eosin.

Result: Three females aged 50 years, 65 years and 72 years presented with peri-umbilical nodules of 3-5 months duration. The first female had associated matted right axillary and inguinal lymph node enlargement and had pelvic surgery which was not subjected to tissue histology in a peripheral hospital 5 months prior to presentation. The second patient's nodule was ulcerated and foul smelling and she also had a 16 week sized uterine mass while the third had total abdominal hysterectomy a year prior to presentation in our hospital for endometrial carcinoma. Tissue specimens sent for histology showed malignant glandular tumours with focal papillary and cystic patterns. They were composed of columnar cells having hyperchromatic nuclei and abundant cytoplasm and were diagnosed metastatic adenocarcinoma with likely primary from the endometrium or ovary.

Conclusion: Peri-umbilical metastasis from endometrial and ovarian adenocarcinoma is uncommon unlike gastro-intestinal tumours which commonly involve the umbilicus (sister Joseph's nodule). However, in advanced disease or late presentaion, a high index of suspicion and the presence of malignant cells conforming morphologically and immunohistochemically with those of the endometrium as in our cases will aid definitive diagnosis.

P42

Struma ovarii presenting with ascites and increased CA 125

P Mairembam[1], R Arora[1]
[1]University College Hospital

Background: The clinical presentation of pelvic mass, ascites and raised CA 125 is most suggestive of a malignant ovarian tumour. Struma ovarii is usually an incidental finding or present with non-specific symptoms such as abdominal pain or abdominal distension. Symptoms of hyperthyroidism occur in fewer than 10% of patients with struma ovarii.

Case Report: A 78 year old woman presented at A&E with shortness of breath. Examination revealed a pelvic mass and ascites. CT abdomen and pelvis showed extensive ascites and an enhancing right adnexal mass. The CA 125 was increased at 1900. CT chest showed no hydrothorax. The clinical impression was that of a malignant ovarian tumour and she underwent hysterectomy with bilateral salpingo-oophorectomy.
Histology showed ovary containing lobules of mature thyroid tissue. The thyroid follicles showed varying sizes, some contained thick colloid and were lined by cuboidal cells with no evidence of papillary architecture or nuclear grooves. No other teratomatous or immature elements were seen. Immunohistochemistry showed positivity for thyroglobulin, TTF-1 and patchy positivity for CD56.

Conclusion: Struma ovarii although benign can present with clinical findings mimicking a malignant tumour in some instances. Our case is unusual due to the age and the clinical presentation of struma ovarii. There have been case reports of struma ovarii presenting as pseudoMeigs' syndrome and elevated CA 125. The term atypical pseudoMeigs' syndrome has also been used for a case of uterine leiomyoma presenting with ascites and elevated CA 125 without hydrothorax.

P43

The importance of expert central review in the diagnosis of lymphoid malignancies in a regional cancer network

I Proctor[1], C McNamara[2], M Rodriguez-Justo[1], P Isaacson[1], A Ramsay[1]
[1]Department of Histopathology, University College London Hospital
[2]Department of Haematology, Royal Free Hospital

In 2003 centralised specialist review of lymphoma was established within the North London Cancer Network. All biopsies suspicious for lymphoid malignancy were reviewed centrally by nationally-recognised experts in lymphoma pathology. This study assesses the impact of this specialist review on diagnostic accuracy and patient management. The network includes 6 Trusts and from 2003 to 2008 1,949 cases were submitted for review. A documented diagnosis was included in 1,873 cases (96.1%).

The overall rate of discordant diagnoses was 26.3%; different lymphoma types showed rates ranging from 2.1% to 72.4%. The discordance rates for the most common diagnoses were:

	n	Discordance rate (%)
Diffuse large B-cell lymphoma	515	29.9
Follicular lymphoma	335	27.8
Plasma cell myeloma	191	2.1
Classical Hodgkin lymphoma	185	7.0
Chronic lymphocytic leukaemia	176	21.6
Lymphoplasmacytic lymphoma	88	39.8
Reactive changes	47	29.8
Mantle cell lymphoma	44	34.1

The potential effect of discordant diagnoses were classified as: (A) none/minor clinical relevance (17.9%), or (B) serious impact on patient care (80.5%). Discordance rates decreased over time, reflecting increased accuracy of the submitted diagnoses.

In conclusion, expert review of lymphoma cases in North London resulted in re-diagnosis of 513 cases over 5 years (26.3%). In 413 cases (80.5%) this had a potentially significant impact on patient care. Whilst local diagnostic skills have improved, there remains a need for central expert review in lymphoma pathology.

P44

The histopathology of DNA and adenoviral vaccination

A Gilchrist[1], K Colbjørn Larsen[2], MG Cottingham[2], J Furze[2], DH Wyllie[2], AVS Hill[2], **EJ Soilleux**[1]
[1]Department of Cellular Pathology, John Radcliffe Hospital, Oxford
[2]The Jenner Institute, University of Oxford, Oxford

In general, non-replicating adenoviral vectors are more immunogenic than DNA vectors, although both are components of phase I/II next-generation vaccine regimes. Since little is known about the immunological features of the vaccination sites and draining lymph nodes, we investigated this histologically. Female Balb/c or C57BL/6J mice were immunised in the tibialis muscle with either a plasmid or adenoviral vector expressing a model antigen; in adenoviruses, the antigen was fused to green fluorescent protein. Some vectors also expressed experimental molecular adjuvants from the same construct. Injection of vehicle (phosphate buffered saline) was used as an additional control. Quantitative immunohistochemical studies were performed on animals sacrificed 1, 2 and 5 days after injection.

All injection sites showed a predominantly macrophage and neutrophil infiltrate with extension through muscle septa and, to a lesser extent, between muscle fibres. Responses to vaccine regimes differed; additionally, a change in infiltration pattern was observed between timepoints. Immune activity appeared highly localized in the injection site, making analysis based on cells/field difficult; optimal quantitation techniques remain to be established. Draining lymph nodes showed variable enlargement, paracortical expansion by T-cells and dendritic cells, and follicular hyperplasia.

This detailed study of injection site and lymph node histology in non replicating vector vaccination shows intense but localized, immune activity following injection. Surprisingly, a clear histology signature of DNA versus the more effective adenoviral injection was not apparent.

P45

The prevalence of in-situ follicular lymphoma in benign lymph node excision biopsies

H El-Daly[1], P Rao[1], K Patel[1], H Ibrahim[1], K Naresh[1]
[1]*Histopathology Department, Imperial College of Science, Technology and Medicine, London.*

An in-situ lymphoma can be conceptualized as presence of clonally expanded lymphoid populations within the confines of specific compartments of lymphoid organs, and these compartments correspond to the normal location of neoplastic cell's benign counterparts. In-situ localization of follicular lymphoma was first described in 2002 by Cong et al. The aim of this study was to investigate to prevalence of in-situ follicular lymphoma in benign lymph nodes received between 1992-2009. Benign lymph nodes included in cancer resection specimens and lymph node biopsies associated with infections were not included in this study. A total of 230 cases were included in the study, and this included 5 cases where a diagnosis of in-situ lymphoma had been made at diagnosis. On screening the BCL2 immunostained slides, we identified a total of seven cases (including two cases not identified previously), which showed strong Bcl-2 staining of follicle centre cells within the germinal centres. These cells also expressed CD10 and BCL6, thus confirming a follicle centre origin. The data shows the prevalence of in-situ follicular lymphoma to be 3%. This is the first study to describe the prevalence of in-situ follicular lymphoma in 'benign/reactive' lymph nodes. We are currently performing cytogenetic and molecular analysis on a subset of these cases to gain insight into the pathophysiology of these interesting lesions of which the clinical significance remains uncertain.

P46

Multicentric Castleman Disease – subgroup with enhanced tissue-T-Cell response: is there a role for follicular dendritic cells?

H El-Daly[1], M Bower[2], K Naresh[1]
[1]*1Histopathology Department, Imperial College of Science, Technology and Medicine, London, [2]Department of Medical oncology and HIV Medicine, Chelsea Westminster Hospital, London.*

Multicentric Castleman Disease (MCD) is a rare human herpes virus type 8 (HHV8) associated lymphoproliferative disorder that occurs more frequently in patients infected with human immunodeficiency virus (HIV). In tissue samples, the mantle zones are expanded and contain variable numbers of larger cells with moderate amount of amphophilic cytoplasm, a large vesicular nucleus and a prominent nucleolus. These cells, termed plasmablasts are infected with human herpes virus type 8 (HHV8) and show expression of latent nuclear antigen 1 (LANA1), express IgM heavy chain and are monotypic for lambda light chain. We revisited the immunohistological features in 25 lymph node (LN) and three spleen samples of MCD. In 5 (20%) LN and 1 (33%) spleen sample, HHV-8 latent nuclear antigen 1 (LANA-1) staining was also noted on the follicular dendritic cells (FDCs). The HHV8-positive-FDC subgroup of cases had significantly higher numbers of CD3-positive T-cells infiltrating the follicles as compared to the HHV8-negative-FDC subgroup (p=0.045). Furthermore, the numbers of HHV-8 positive plasmablasts and serum HHV-8 viral copy numbers were lower among the HHV8-positive-FDC subgroup as compared to the HHV8-negative-FDC subgroup. Our findings show, for the first time, possible presentation of an HHV-8 antigen by FDCs in MCD.

P47

An audit of the role of core biopsies in the diagnosis of lymphoma

P Bhagwat[1], B Newell[1]
[1]*St George's Hospital, London*

Introduction: Core biopsies of deep lymph nodes can provide a mechanism for obtaining a diagnosis without invasive surgery. However, the diagnosis of lymphoma can be difficult in small cores that do not permit assessment of the whole nodal architecture. The purpose of this audit was to determine if core biopsies offer a useful means for diagnosing lymphoma.
Method: All the lymph node biopsy reports from 2004 to 2008 were retrieved by a SNOMED search. These reports were inspected to identify the core biopsies.
Results: Of the 5836 lymph node specimens, 44 were core biopsies for suspected lymphoma. Thirty-four were diagnostic (77%). There were 17 lymphomas, (14 non-Hodgkin, 3 Hodgkin) and 2 acute leukaemias, nine normal lymph nodes, five cases of granulomatous inflammation and one of chronic inflammation; none of these last fifteen patients had further specimens available locally. Two of the 10 non-diagnostic specimens underwent another procedure: one patient had marginal zone lymphoma; the other had diffuse large B cell lymphoma.
Conclusion: Just over three-quarters of lymph node core biopsies for suspected lymphoma yielded diagnostic material. Although a 23% chance remains of obtaining a non-diagnostic specimen, the complications of a second procedure in these patients may be offset by the benefits to the three-quarters of patients who are spared open surgery. Further studies may determine if our local results are generalisable. In order to avoid giving unrealistic expectations it is also important to caution patients that the diagnostic failure rate is around 23%.

P48

Acute Rheumatic Fever – is it really rare in the western world?

A Merve[1], L Kiho[1], S Baithun[1]
[1]*Barts and the London NHS Trust, London*

Introduction: Acute rheumatic fever (RF) is an immunologically mediated multi-system disease, a sequel to group A β-haemolytic streptococcal pharyngitis, manifesting mainly as polyarthritis (75%) and carditis (25-30%). In developing countries RF is endemic and remains one of the major causes of cardiovascular disease and mortality in young people. In developed countries RF is rare with a mean annual incidence fallen to less than 1 per 100,000 population.
Cases: We discuss two unsuspected cases presenting in a span of 6 months. A 5 year old Caucasian male and a 33 year old female, in both of whom the autopsy concluded RF as the cause of death. The common autopsy findings to both cases were erythematous skin rash and fibrinous pancarditis. The second case had vegetations on the aortic valve, while the first had no valve abnormalities. Histologically the myocardium in both cases showed Aschoff bodies, Anitschkow cells and multinucleate cells, with foci of interstitial necrosis. The first case also had Aschoff nodules in the diaphragm.
Discussion and Conclusion: The presence on histology of both Aschoff bodies and Anitschkow cells are pathognomonic for RF. In both cases RF was only confirmed at autopsy by histology. We therefore emphasise the importance of tissue sampling at autopsy. Although thought to be rare, we suggest that RF should be considered during the autopsy in both children and adults, even in unsuspected cases. Further epidemiological studies may be needed to evaluate a possible emerging incidence.

P49

Histopathological changes in the lungs resulting from fatal swine influenza infection

M Green[1], M Sheaff[1], I Ushiro-Lumb[1], L Kiho[1], S Baithun[1]
[1]Cellular Pathology, The Royal London Hospital

Background: A novel swine-origin influenza A virus [SO-2009 A(H1N1)] was identified in March 2009 in Mexico. It has since spread around the globe as a pandemic. A small proportion of individuals develop pneumonia and respiratory failure, with fatalities being more common in individuals with certain pre-existing co-morbidities.
Aims: To describe the histopathological changes found in the lungs of patients who have died following infection with swine influenza virus.
Methods: Four recent post-mortem cases from Greater London were identified, all having tested positive for Influenza SO-2009 A(H1N1) by real-time polymerase chain reaction (RT-PCR) analysis of respiratory specimens. A panel of 10 other respiratory viruses was negative by RT-PCR and microbiology cultures were also negative.
Results: In all cases, swine influenza was found to have been the significant causal factor in the death. Three patients had underlying medical conditions. The fourth patient had no previous medical history but had been taking long-term anabolic steroids. The sections of lung tissue from each case showed changes of acute lung injury.
Conclusions: These cases provide several interesting learning points but most notably an insight into the histopathological changes seen in the lungs with fatal swine influenza infection.

P50

Coronial autopsy tissue: a logistic nightmare

VLJ Salter[1], M Osborn[1]
[1]Imperial College Healthcare NHS Trust

Background: The implementation of 'The Coroner's (Amendment) Rules 2005' and 'The Human Tissue Act 2004' has had a direct impact on the governance of autopsy practice. It is an offence to hold tissue unless explicit consent is given.
Aims: To determine how well one histopathology department complies with these regulations and how adequately tissue, obtained at coronial autopsies, is managed. Methods: From June 2005 until December 2008 the reports of all coronial autopsies undertaken were read. Those autopsies where tissue was taken for histology were identified. Those Coroner's Tissue Retention Forms received, detailing instructions for the appropriate management of the tissue in each case, were then reviewed and all autopsy tissue in the department evaluated.
Results: Definite knowledge that the Coroner's functions had ceased followed by the subsequent appropriate management of tissue occurred in only 10% of cases. In 17% of cases it is not known if the Coroner's functions have ceased and it is therefore impossible to dispose of tissue as required. In 31% of cases no instructions as to the appropriate management of tissue have been received at all.
Conclusions: The fundamental problem is that carrying out the instructions, where present, is dependent on knowing when the Coroner's functions have ceased. At present, the only way to determine this is to repeatedly contact the Coroner's officers: a logistic nightmare!

P51

Spontaneous coronary artery dissection: a poorly understood cause of sudden death in young adults

M Koenig[1], DA Wright[1]
[1]Brighton & Sussex University Hospitals NHS Trust

Coronary artery dissection most frequently occurs as a complication of coronary instrumentation. However, spontaneous coronary artery dissection (SCAD) is a well recognized but rare cause of acute coronary syndrome in young adults and may cause sudden death.

Classically, it is described in peri-partum women but it is increasingly diagnosed in different clinical settings. Aetiology and pathophysiology are poorly understood. Awareness and early recognition are vital to ensure optimal management.

We present the case of a 39 year old woman with a history of mild asthma and hypertension, who was found dead unexpectedly. She was not pregnant or post-partum. Autopsy suggested thrombosis of the distal left anterior descending coronary artery but there was no coronary atherosclerosis. There were no other significant findings at post mortem.

Microscopy revealed haemorrhage between the tunica media and adventitia with associated peri-adventitial eosinophilic infiltrate. Frank vasculitis was not present. Immunofluorescence and enzyme-linked immunosorbent assay proved anti-neutrophil cytoplasmic antibodies, proteinase-3 and myeloperoxidase antibodies to be absent. Plasma total IgE was within the reference range. Routine toxicology was negative.

These findings are typical of SCAD and militate against other associations of dissection such as coronary artery vasculitis as in Churg-Strauss syndrome or cocaine use. There was also no known history of a connective tissue disorder.

We present and discuss our findings in detail and offer a review of the current literature.

P52

The adequacy of the information provided for Coroner's post mortem cases

Y Alwahab[1], P Gallagher[2]
[1]Hammersmith Hospital, [2]Southampton General Hospital

It is very often that a pathologist feels that the information available on the coroner's post mortem fax sheet is not enough and does not provide a full outline of the deceased's medical or drug history.
In this study, we examined 50 post mortem cases performed at Southampton general hospital's mortuary between August 2008 and December 2008. We assessed the information provided on the coroner's fax sheet and compared it with the findings at the post mortem examination and the cause(s) of death. In addition to this, we used Southampton hospital electronic record system (e-docs) as an alternative source of information based on the deceased's previous inpatient and outpatient attendance and GP correspondence. We found that in 20% of cases, the information provided was inadequate and did not relate to the post mortem findings or the cause(s) of death. In 10% of cases, additional useful information was found in the e-docs system that was directly related to the final cause of death. This small study shows that a significant number of coroner's post mortem requests do not provide important information in the deceased's medical history that is present otherwise in other systems e.g. hospital medical notes. We like to stress that adequate information should be made available prior to a post mortem examination. Possible ways to gain additional information would be, GP summary notes, hospital paper notes and hospital electronic record systems.

P53

The reporting of colorectal cancer at King's College Hospital – our experiences over the last two years

R Cheong[1], **S Saw[1]**, H Li[2]
[1]William Harvey Hospital, Ashford, Kent, [2]Kings College Hospital, London

Background: The royal college introduced guidelines for the reporting of colorectal cancer resection specimens in 2007 that included standards for lymph node numbers (12), frequency of serosal involvement (20% colon, 10% rectum) and extramural vascular invasion (>25%).

We look at the reporting of colorectal specimens at King's College Hospital and compare our findings with the standards set by the college.

Methods: All colorectal resection specimens were audited in 2007 and 2008, consisting of 56 colon and 55 rectal specimens.

Results:

	Lymph node number	Serosal involvement	Extramural vascular invasion
colon 2007	19.2	32.0	40.0
colon 2008	17.1	16.7	35.0
rectum 2007	13.4	25.9	40.4
rectum 2008	17.7	7.7	37.0
Average colon	18.2	24.5	38.5
Average rectum	15.6	16.8	37.9

Conclusions: Our overall figures compare well with the current guidelines. Although the incidence of serosal involvement in 2008 is lower than the suggested level, the proportion of patients treated as being involved, would be comparable, as a further 6.7% and 7.7% respectively of colonic and rectal specimens showed tumour within 1mm of the serosa, and would have been treated as serosa positive.

P54

Graduate medical students outperform school leavers, at least in the early years of the course

E Jaynes[1], SA Rushworth[1], PJ Gallagher[1], BG Marshall[1], J Field[1]
[1]School of Medicine, University of Southampton

Many Medical Schools in the United Kingdom have programmes specifically designed for students with a first degree in a subject other than Medicine. In the first two years of our four year Graduate course 40 students follow a separate programme in which all learning is structured around clinical topics and triggered by case studies. There are defined Pathology learning outcomes and one Consultant Pathologist has specific responsibility for coordinating Pathology teaching. There is also enhanced clinical experience in a nearby District General Hospital.

These students join with 200 school leavers in Year 3 and have identical rotations. A demanding end of year examination assesses all of the first three years work. This examination includes problem solving written questions and an OSCE. In each of the first three years of this new programme graduate students scored significantly higher marks in both components of this examination. In the most recent OSCE examination the mean ± SD marks were 4.87 ± 0.49 vs 4.30 ± 0.56 (graduates vs school leavers; maximum possible score 6) and in the written examination $72.6 \pm 6.5\%$ vs $63.7 \pm 8.2\%$, both $P < 0.001$. Two cohorts of graduate students have now qualified. The first group had significantly higher marks than school leavers in the final examination, but the second group did not. These results demonstrate for the first time that graduate students have a superior all round performance at least in the early years of the course.

P55

Analysis of incident reports in cellular pathology service as an important component of the continuous quality improvement process

S Sen[1], E Rakha[1], L Bakowski[1], S O'Connor[1]
[1]Nottingham University Hospitals

Incident reporting forms an important component of the continuous quality improvement process (QIP). Characterizing the nature and causes of adverse events is important for risk management and resource allocation, but published data on incident reports in cellular pathology remains scanty. In this study, we analysed all incidents and adverse events reported in our department over a 7-month period over 2 consecutive years. Our aim was to classify incidents by significance and into categories that could facilitate their root cause analysis and guide the institution of effective countermeasures.

Results: In 2009, 131 incidents were reported compared to only 23 incidents in the same period in 2008, an increase which may in part have reflected the appointment of a dedicated quality manager in 2009. The majority (84) occurred in the pre-analytic phase of the laboratory process, with 29 in the analytic and 18 in the post-analytic phases. Booking-in related incidents were most common (63 cases). Most incidents (99) posed potential harm, whereas 31 caused actual patient harm. Only 20 cases posed a major risk, whereas 24 were associated with moderate and 71 with minor risk. All incidents were graphed according to frequency on a Pareto plot, enabling identification of a "significant few" for further detailed analysis. Formal root cause analysis was carried out for these using Ishikara diagrams, enabling identification of underlying problems. Effective countermeasures were then put in place where possible. In conclusion, analysis of incident reports forms an important component of QIP in cellular pathology.

P56

Audit on malignant melanoma in public and private medicine in Ireland

J Cryan[1], F Al Halban[2], M Leader[1]
[1]Department of Pathology, Royal College of Surgeons Ireland, [2]Royal College of Surgeons Ireland

A strong social gradient is documented in many types of ill-health, in that people from more deprived areas experience more illness than those in more affluent communities. However, this is not the case for malignant melanoma incidence and mortality rates. Access to healthcare remains a key reason for having health insurance. This audit set out to compare malignant melanoma in private and public healthcare in Ireland. Histopathology reports of melanoma from 2005-2008 from a public laboratory and a private laboratory were examined for variables including age, sex, site, melanoma subtype, and Breslow thickness. 268 reports were included (145 private, 123 public). Most cases (56%) occurred in the 50-79yrs age group. Female cases outnumbered male cases (55% vs 45%). Superficial spreading melanoma was the most common subtype (32%). 49% cases presented with a Breslow thickness of 1mm or greater. The most striking difference between the public and private institutions was that 59% of cases in the private institution presented with a Breslow thickness <1mm as compared with 41% in the public institution (p<0.05). This audit demonstrates a significant difference between public and private institutions with respect to Breslow thickness, a universally accepted prognostic indicator in malignant melanoma. There are two possible reasons for this; lack of awareness in public patients, or longer access time to diagnosis for public patients. This requires further study. Increasing the rate of early detection must be improved and rapid access to diagnosis is essential for all patients.

P57

Cytology and post-mortem training in ST1 Trainees – is it effective? Are there changes to be made?

I Salama[1], S Cossins[2], AH McGregor[3]
[1]Wexham Park Hospital, [2]National Histopathology Training School
[3]Leicester Histopathology Training School

Aim: To asses the adequacy of cytology and post-mortem (PM) training of ST1 trainees in the UK.

Method: A structured, anonymous questionnaire was sent to all ST1 trainees in all deaneries by e-mail, asking about key elements of their cytology and PM training. Trainees were also asked, based on their first year of training in these subjects, whether they would consider doing gynaecological cytology or PM light training when possible in 2010. Returned questionnaires were analysed.

Results: 59% of all questionnaires were returned and analysed. Only 9% of all trainees had any previous experience in cytology prior to starting ST1 training. Training in both gynae and non-gynae cytology varied between deaneries with some trainees gaining 8 weeks experience and others only 1 week (average 3.7 and 3.9 weeks respectively). Only 76% of trainees felt confident in gynae cytology and even less (64%) in non-gynae cytology. 19% of trainees felt they would consider doing cytology light training.

38 % of trainees had previous experience in PMs. 86% of trainees had completed their first year target of 20 PMs. 91% of trainees felt confident performing PMs, however only 32% felt confident looking at PM histology. 30% would consider PM light training.

Conclusion: Cytology and PM training appears to be sub-optimal; we suggest strategies to enhance training.

P58

Audit of skin cut-up since implementation of extended role of biomedical scientists in specimen description, dissection and sampling

E Husband[1]
[1]William Harvey Hospital, Ashford, Kent

Implementation of the extended role of biomedical scientists (BMSs) was intended to facilitate consultants with high volume of workload, whilst maintaining quality of care. The aim of this audit was to provide evidence of fulfilment of this intent. The objective was to assess macroscopic descriptions in order to show BMS skin cut-up meets set departmental standards.

Overall (mean) adherence to set departmental standards was calculated at 98.8%, evidence that high quality care has been maintained. Of 206 skin specimens assessed, no significant errors were identified.

Only one specimen was unsuitable for BMS cut-up, however, was processed according to protocol and did not result in an erroneous final report. BMSs are not currently trained to cut-up skin specimens in categories of high complexity. Given the high quality of cut-up demonstrated there are good arguments to extend the role of BMS staff.

A suitable macroscopic description is essential to the reporting pathologist who has not seen the specimen. External appearance was unrecorded for 11 specimens. However, not all descriptive features have equal importance and tighter adherence to certain parts of protocol may not necessarily impact upon accuracy of final report. Regular feedback from reporting pathologists to dissectors regarding specimen handling is thus recommended.

Volume of work within the department continues to expand therefore biomedical scientist cut-up is vital to working practices; reducing pathologist specimen handling time and increasing pathologist reporting time.

P59

An audit of thyroid fine needle aspiration cytology reporting in a tertiary referral centre

E Walding[1], A Chandra[1], M Masood[1]
[1]Department of Histopathology, Guy's & St Thomas' NHS Trust

Introduction: Fine needle aspiration cytology (FNAC) is the predominant means of obtaining thyroid material for preoperative microscopic analysis. In the UK thyroid FNAC reports are classified as: Thy 1 - inadequate; Thy 2 - benign; Thy 3 - equivocal/follicular lesion; Thy 4 - suspicious of malignancy; Thy 5 - malignant.

Aims: To assess the quality of thyroid FNAC reporting at a tertiary referral centre against local and international guidelines.

Methods: All patients who underwent thyroid FNAC, and outside cases received for specialist opinion, over a 12 month period were included. The data collected included the initial FNAC category, the highest category of repeated FNAC, and histological correlation where applicable.

Results: Of the initial FNACs 9% were Thy 1 and 16% were Thy 3. Only 2% of repeat FNACs identified a previously undiagnosed malignancy. Histological correlation showed: Thy 1 = 0% malignant; Thy 2 = 5% malignant; Thy 3B = 11% malignant; Thy 3A = 35% malignant; Thy 4 = 50% malignant; Thy 5 = 100% malignant.

Discussion: The number of inadequate FNACs was below the local target of <15%. Conversely 16% of FNACs were equivocal - improved over previous cycles but above the local target of <15%. The risks of malignancy per Thy category were in line with those published internationally. However the numbers in our series were small, limiting accuracy. Further audit cycles are needed to increase the sample size, assess the effects of implemented changes and ensure quality improvement.

P60

Socioeconomic factors and characteristics of breast cancer

D Shahid[1], M S Bashir[2]
[1]Medical School University of Birmingham, [2]Salford Royal Hospital

A number of tumour characteristics affect prognosis and treatment of breast cancer. This study was carried out to see if breast tumour characteristics vary in two groups of socioeconomically different patients .

Data was collected from the Telepath system within the Histopathology department . Details of the grade, size, hormone receptor status and HER-2 receptor status were found for 108 NHS patients and 146 private sector patients. The NHS hospital has a economically deprived and the private hospital an affluent catchment population.

16% of NHS patients had grade 1 tumours, 55% grade 2 and 28% grade 3. This was 18%, 60% and 20% respectively in the private sector. 37% of NHS and 13% of private patients were ER/PR-ve .92% of NHS and 91% of private patients had HER-2 negative tumours. 70% of private and 23% of NHS patients had a T1 tumour. 30% of private and 67% of NHS patients had a T2 tumour.

There was a significant difference in the size of the tumour between the two groups with the patients in the private sector having a higher percentage of T1 tumours at surgery. If you compare tumours that are ER and PR negative then private patients have significantly less receptor negative tumours.

No significant difference in tumour grade was seen in the two groups.This is in keeping with previous studies which have demonstrated that patients in affluent groups have smaller tumours, that are more often receptor positive.

P61

An audit of B3 and B4 categories in needle core biopsies from screening and symptomatic breast services in the West of Ireland

H Ingoldsby[1,2], PJ Gleeson[1], C Inderhaug[1,2], M Sheehan[1,3], G Callagy[1,2]

[1]Department of Pathology, Galway University Hospitals, [2]Division of Pathology, NUI Galway, Clinical Science Institute, [3]BreastCheck Western Unit, Galway University Hospitals

We audited biopsies coded B3 (uncertain malignant potential) and B4 (suspicious for malignancy) from the breast screening service (BSS) and symptomatic service (BSXS) in the West of Ireland.

All B3 and B4 biopsies from BSS (15 months) and BSXS (24 months) and subsequent excisions were reviewed.

The B3 category was uncommon in both BSS and BSXS (n=62, 11% and n=67, 4% respectively). The B4 category was less common in BSS (n=14, 2.5%) and was rarely made in BSXS (n=2, 0.1%). B3 cases were as follows:

Diagnosis	BSS n= (%)	BSXS n= (%)
AIDEP	23 (37)	24 (36)
Fibroepithelial lesion	2 (3)	16 (24)
Lobular neoplasia	7 (11)	4 (6)
Papillary lesion	10 (16)	16 (24)
Radial scar	19 (31)	6 (9)
Other	1 (2)	1 (1)
Total	62 (100)	67 (100)

B4 cases from the BSS comprised biopsies suspicious for DCIS (n=13) and for tubular carcinoma (n=1). Two B4 cases from the BSXS were suspicious for DCIS. The positive predictive value (PPV) for B3 lesions in the BSS and BSXS was 13% and 7% respectively. The PPV for B4 lesions in BSS and BSXS was 79% and 50% respectively.

B3 and B4 categories are more common in the BSS than BSXS. The frequency of specific diagnoses within each category varies between both services. Overall PPV for both categories was higher in the BSS. The observed differences in results may be partly explained by variations in the clinicoradiological features of lesions presenting to each service.

P62

Spindle cell lesions of the breast – an example of a case and review of literature with emphasis on approach to interpretation

M Abdul Aziz[1-2], M Sheehan[1-2]

[1]BreastCheck Western Unit, Galway University Hospitals, Galway, Ireland, [2]Department of Pathology, Galway University Hospitals, Galway

Introduction: Spindle cell lesions of the breast encompass a heterogeneous group of entities. These may be reactive or neoplastic, which in turn may be benign or malignant. The interpretation of a spindle cell lesion in a core biopsy is challenging and a systematic approach is needed to avoid misinterpretation. **Case history**: A 52 year-old woman was referred to BreastCheck Western Unit where a circumscribed lesion was identified in right upper inner quadrant of the breast. Biopsy showed a bland spindle cell lesion with trapped fat spaces and absent breast ducts and lobules. Perivascular lymphocytes are also noted. A panel of immunohistochemistry was performed and the features were felt to be suggestive of a myofibroblastoma. Subsequent resection confirmed the diagnosis. **Discussion**: Spindle cell lesions of the breast represent a diagnostic challenge and have been a subject of interest for pathologists. We illustrate a case of a spindle cell lesion of the breast which was picked up by screening mammography. We performed a literature review looking at the differential diagnoses that need to be considered when one encounters a spindle cell lesion in the breast, with a suggested approach to core biopsy interpretation of these lesions. The role of immunohistochemistry is also discussed.

P63

Viewing, encoding and analysing the mammary forest

JJ Going[1]

[1]University of Glasgow

Trees and their properties have been investigated in fields from hydrology to computer science. This study explored the possibility of uniquely encoding the branching of a complete mammary lobe as a binary tree (a tree with no vertex of degree >3). Mammary duct branching is modified by abnormal expression or mutation of genes including myc and BRCA1. Motivation was to develop tools describing branching of human mammary duct systems, given that human breast cancer risk is conditioned by very early life events, which might leave traces in terms of abnormal branching. Previously, branching of all duct systems (lobes) in one complete breast was traced through serial subgross sections. The largest lobe was chosen and 3D Cartesian coordinates of all identifiable branch points were captured and the edges linking branch points were recorded. A 3D model of lobe branching created from this data has now been reduced to a (planar) binary tree in canonical form. All edges were assigned their Strahler number = S. Beginning at the root, the 'child' with lower S was placed to the left. If Strahler numbers were equal, inequality at deeper levels was used. This showed large differences in the depth of branching between subtrees. Following canonicalization all vertices were numbered in a breadth-first manner, and finally the tree was captured as its Prufer code, from which the entire tree can be reconstructed and for which analytical techniques exist. This study establishes the feasibility of capturing information about breast duct branching in a form suitable for further analysis from subgross sections of breast tissue.

P64

Translocation of the Ret oncogene in human primary breast cancer

K Unger[1], K Patel[1], RCF Leonard[1], GA Thomas[1]

[1]Imperial College London

The Ret oncogene is known to be activated in papillary thyroid cancer (PTC) by gene rearrangement More recently activation of c-Ret has been demonstrated in breast cancer by expression analysis. However, this technique only identifies activation of the 5' end of the gene and therefore activation of the 3' end of the gene by translocation, as is the case in PTC, would not be identified. The aim of this project was therefore to identify Ret gene rearrangement in breast cancer. RNA from 43 unselected cases of breast cancer were sourced from the Wales Cancer Bank (www.walescancerbank.com) . Primers located in exons 2/3 (extracellular: EC) and exons 12/13 (tyrosine kinase, TK) were used to identify expression of the Ret oncogene. 5 (16%) cases showed balanced expression of Ret, suggesting expression of the full length transcript. 9 (28%) showed expression of the TK domain only and 3 (9%) further cases showed expression of the EC domain, suggesting translocation of the 3' and 5' ends of the gene respectively. 15 cases were negative for any form of expression of the Ret gene. Rearrangement specific qRT-PCR showed that the rearrangements found in thyroid cancer (PTC1 and 3) were not found in breast cancer. Ret rearrangements were also more commonly found in higher grade disease (G3vsG1: 6 vs 1), but in this small study we were not able to show correlation with ER or Her2 status. Targeting the Ret receptor may therefore have a role in the treatment of breast cancer.

P65

Membraneous staining of the key Adherens Junctional (AJ) Protein Alpha-T-Catenin (CTNNA3) is compromised in human breast cancer

G Woods[1], J Smith[1], A O'Grady[2], MH Meehan[1], D O'Connor[3], O McCormack[1], B McGrogan[1], M Harrison[4], MJ Kerin[5], E Kaye[2], A McCann[1], S Phelan[1]

[1]School of Medicine and Medical Science (SMMS) and Conway Institute of Biomolecular and Biomedical Research, University College Dublin (UCD), Ireland, [2]Department of Pathology, Royal College of Surgeons in Ireland (RCSI), Ireland, [3]School of Biomolecular and Biomedical Science (SBBS) and Conway Institute of Biomolecluar and Biomedical Research, University College Dublin (UCD), Ireland, [4]Department of Pathology, Mater Misericordiae Hospital, Ireland, [5]Department of Surgery, Clinical Science Institute (CSI), University College Hospital Galway (UCHG), Ireland.

The purpose of this study was to immunohistochemically (IHC) analyse the expression of alpha-T-catenin (CTNNA3), a key adherens junctional protein, in breast cancer samples in which the primary tumour (T), matched tumour associated normal (TAN) and paired lymph node metastasis (LNM) were available for analysis, totalling 66 tissue samples. In addition CTNNA3 localisation was assessed in hypoxic versus non-hypoxic regions of the primary tumours identified through CAIX immunoreactivity.

IHC quantification was carried out on a breast tissue microarray (TMA) platform representing quadruple cores of 112 breast cancer patients following scanning into the Aperio Automated Imaging Platform. Twenty-one of these patients had T, TAN and LNM available for IHC analysis. Manual quantification of both cytoplasmic and membraneous staining for CTNNA3 was carried out and consistently tumour associated normal (TAN) (18/21) tissue demonstrated crisp intense membraneous staining for CTNNA3 with a reduction in membraneous staining in the tumour cells, independent of CTNNA3 loss of heterozygosity (LOH). There was variable re-expression in the LNM (8/18). Finally, hypoxic tumour regions were identified by crisp intense membraneous staining for the hypoxic marker Carbonic Anhydrase 9 (CA9). Compromised membraneous localisation of alpha-T-catenin (CTNNA3) was observed in hypoxic regions.

In conclusion, membraneous localisation of CTNNA3 is compromised in primary breast cancers and can demonstrate a relocation to the membrane in paired lymph node deposits. CTNNA3 localisation may also be subject to a hypoxic tumour microenvironment.

P66

Significance of atypical intraductal epithelial proliferative lesions diagnosed on needle core biopsies of the breast

BC Ho[1], EA Rakha[1], V Naik[1], S Sen[1], Z Hodi[1], IO Ellis[1], AHS Lee[1].

[1]Histopathology Dept, Nottingham City Hospital

Introduction: Breast lesions showing atypical intraductal proliferation (AIDEP) are increasingly recognised on needle core biopsy (NCB), with ongoing debate on their best management. We present our recent experience with AIDEP lesions correlated with follow-up excision biopsy findings.
Methods: B3 (AIDEP) lesions diagnosed over a 2-year period were reviewed by 3 breast pathologists blind of subsequent results. NCB pathology findings were correlated with excision biopsy results in 43.
Results: AIDEP lesions comprised 45/155 (29%) B3 lesions, with an overall positive predictive value for in situ and/or invasive malignancy (PPV) of 44% (19/43). An ADH-like pattern constituted the majority (67%) of AIDEP lesions. Malignancy on excision was found as follows: apocrine atypia (0/2), pure flat epithelial atypia (FEA) (1/7), FEA+lobular neoplasia (0/2), FEA+ADH-like areas (3/7), ADH-like areas only (13/23) and scant atypical epithelial cells separate from the core (2/2). The single upgraded case of pure FEA was biopsied for screen-detected calcifications, showed radiologic-pathologic concordance and revealed low grade DCIS on final histology. As a group, clinically malignant or indeterminate lesions with an AIDEP diagnosis showed a significantly higher PPV for malignancy than the group of clinically benign lesions (78% vs 33%, p=0.04).
Conclusions: Isolated FEA showed a lower PPV (14%) for malignancy than ADH-like lesions (53%), but this was not statistically significant. Larger studies on FEA are needed to investigate if there is a difference in risk and determine optimal management.

P67

High resolution melting analysis for mutation screening of clinical samples

A Benhasouna[1], P Desmond[1], A Green[1], E Rakha[1], I Ellis[1]

[1]University of Nottingham

Introduction: The development of targeted therapies has increased the need for a rapid and robust molecular characterisation of cancers. High Resolution Melting (HRM) analysis is a rapid, sensitive, specific, cost-effective and reliable screening method for gene mutations. However, data on application of HRM to archival clinical samples is lacking.
Aim and method: This study was undertaken to investigate whether HRM could be used to screen for TP53 mutations using six matched fresh frozen (FF) and formalin-fixed paraffin embedded (FFPE) breast cancer tissue samples.
Results: Considerable variation was seen in mutation detection by HRM analysis between samples from FF and corresponding matched FFPE tissues. Excellent agreement was observed between HRM on FF material and gene sequencing results; the same agreement did not apply to HRM on FFPE tissue and sequencing.
Conclusion: DNA from FFPE clinical samples is more degraded than that obtained from FF tissues resulting in higher levels of variation seen in HRM. DNA quality and integrity is a critical factor and we conclude that HRM can be reliably applied to FF tissues for screening the TP53 gene. Additional work is required to modify the technique on FFPE tissue to obtain acceptable sensitivity and specificity.

P68

Molecular phenotypes of breast cancer in different ethnic groups

RL Bowen[1], KA Menezes[1], M Ornstein[2], C Choy[2], SE Slater[2], YW White[2], MMM Dawoud[1], G Elia[1], DA Ryan[3], N Asaad[4], JL Jones[1]

[1]Tumour Biology, Institute of Cancer, Queen Mary University of London [2]Homerton University Hospital, Hackney, London, [3]Department of Pathology, Barts and The London NHS Trust, London, [4]Department of Pathology, Faculty of Medicine, Menoufyia University, Egypt

There are differences in the behaviour of breast cancer in different ethnic groups though the reasons for this are not well established. It is evident that molecular subtypes of breast cancer can influence disease progression and outcome. We hypothesized that clinicopathological variation between ethnic groups may be reflected in the relative frequency of tumour molecular subtypes. This study analysed breast tumours from black women and white British women, and a pilot cohort of Egyptian women, to determine the prevalence of different molecular subgroups using the criteria of Carey et al[1].
The tumours from 122 black women, 140 white women and 21 Egyptian women underwent immunohistochemistry for classifcation into the four main phenotypes (Luminal A, Luminal B, HER2 and Basal/ TN).
Differences were most marked in the young age group (≤ 50 years). Young black women have more than double the frequency of triple negative (TN) tumours, accounting for 32% of cases, compared to their white British and Egyptian counterparts. All TN tumours occurring in younger white women expressed at least one of the classical basal markers (CK5/6, CK14, EGFR), whereas there was heterogeneity in young black women with only 56% of TNs expressing at least one of these markers. Young white and Egyptian women show the highest frequency of Luminal A tumours (>70%).
This study confirms differences in the biology of breast cancers in women of different ethnic origins and may underlie differences observed in the clinical patterns of the disease.
[1] Carey, L et al, JAMA, 2006.

P69

Validation study of 4B5 and CB11 monoclonal antibodies for Her2 testing of breast cancer in a Her2 reference centre

C Ho-Yen[1], G Donald[1], H White[1], D Fish[1], R Liebmann[1]
[1]*Maidstone and Tunbridge Wells NHS Trust*

Introduction: Breast cancers that overexpress Her2 (c-erb-B2) are amenable to treatment with monoclonal antibody to the Her2 protein (trastuzumab). Detection of Her2 overexpression is accomplished through immunohistochemistry (IHC) followed by fluorescence in-situ hybridisation (FISH) on those cases showing equivocal staining. Recent advances in antibody development and automated staining have led to new reagents that may have advantages over established regimens. The aim of this study is to validate the use of two new antibody reagents in comparison to the current Dako HercepTest regimen.

Methods: Sections were cut on 281 consecutive invasive breast cancer cases (referral and local) and stained with Dako HercepTest A085 polyclonal antibody and Leica CB11 monoclonal antibody (using a Dako Autostainer and Leica BondmaX respectively). 274 cases were also stained with the Ventana 4B5 antibody (using the Ventana Benchmark machine). The slides were scored using the updated UK Her2 testing guidelines. The versatility and labour requirements of the different regimens were noted.

Results: 84% of 4B5 and 78% of CB11 cases showed no clinical difference compared to HercepTest. In 11% and 15% (4B5 and CB11 respectively) of cases, HercepTest equivocal cases were scored as negative. The 4B5 regimen required less manual input, was more versatile and showed more intense staining.

Conclusion: Our findings validate the use of 4B5 and CB11 in the detection of Her2 overexpression. 4B5 in particular is easier to use and interpret than HercepTest and may reduce rates of FISH.

P70

Myoepithelial cells in cancer containing breasts exhibit differences in their expression profile

V Modes[1], JA Shaw[1], G Ball[2], **RA Walker**[1]
[1]*University of Leicester, *[2]*Nottingham Trent University*

Myoepithelial cells are important in controlling growth and differentiation and have been described as natural tumour suppressors. We have previously shown that myoepithelial cells from non-involved tissue from cancer-containing breasts (NTCCB) have higher expression of fibroblast growth factor 2 (FGF-2), a modulator of cell survival, when compared to normal breast tissue from age matched women without cancer. Myoepithelial cells were isolated, using positive selection, purity assessed and RNA extracted. 5 myoepithelial cell samples from NTCCB and 6 age-matching samples from normal breast were processed using the Illumina Whole-Genome Expression cDNA Microarray BeadChips. Results were analysed using a stepwise forward selection artificial neural network (ANN) modelling approach and validated using real time qPCR TaqMan assays.

The ANN algorithm ranked genes based on the difference of their expression levels and their predictive value. The top two genes were matrix metallopeptidase 23B (MMP23B), upregulated in NTCCB and sprouty homolog 1 (SPRY1), a FGF signalling antagonist and inhibitor of the Ras/MAP kinase pathway, that was downregulated. Other genes showing differences related to extracellular matrix, members of the tumour necrosis factor superfamily and RAB38. Results from real time qPCR were less significant for MMP23B and SPRY1 but confirmed the higher expression of FGF-2 in myoepithelial cells from NTCCB (p=0.021), as did western blotting.

In conclusion, these data confirm that there are differences in myoepithelial cells in breasts in which a cancer develops, which could affect their tumour suppressor function and so increase predisposition.

P71

Audit of respiratory cytology and histology

N Schneider[1], A Abdulkadir[1]
[1]*Royal Sussex County Hospital, Brighton, *[2]*William Harvey Hospital, Ashford, Kent*

Retrospective, single site audit from July 2007 to June 2008 at the William Harvey Hospital (WHH) to assess the accuracy of respiratory cytology and compare its performance to previous years and other departments.

All respiratory cytology specimens (sputa, bronchial washings, brushings and fine needle aspirations (FNA)) were collated with their histology. Biopsy was considered the gold standard.

A total of 424 respiratory cytology and 191 biopsy specimens were examined. Although FNA was the least often used method, it was the most accurate. Sputum cytology, the most frequently used method was the least sensitive. In 42 cases divergent diagnoses in histology and cytology were identified. Factors leading to discrepancies were poor slide quality and unrepresentative sample.

FNA is the most sensitive sample method but costly and invasive, hence only used in cases strongly suspicious of malignancy.

Sputum samples, showing the lowest sensitivity, are readily taken and also utilised in conditions other than neoplasm.

The performance of WHH is in line with previous audit cycles and compared to other departments. Low sputum sensitivity might be due to "single sampling" instead of the recommended three consecutive samples. Reinforcement of this practice could improve results.

Since most discrepant cases were still negative on review, malignancy was not necessarily missed but may not have been present in the sample. On-site assessment of cytology sampling could improve adequacy and quality of samples.

P72

Ethnic differences in the vascular compliance of healthy young adults

I Naeem[1]
[1]*St Bartholomew's and the Royal London School of Medicine and Dentistry*

Background: Arterial stiffness is a powerful predictor of illness and death from cardiovascular disease and interest in its routine measurement is steadily increasing. Migrant South Asians (SA) have a 3-5 fold increase in the risk for myocardial infarction and cardiovascular mortality and many high risk conditions, such as hypertension, are known to have an ethnic dimension. Therefore ethnic differences in arterial stiffness merit further study. We investigated the effect of ethnicity on arterial stiffness by measuring arterial pulse wave velocity (PWV) in a group of young, healthy SAs and their Caucasian counterparts.

Subjects and Methods: Pulse transit time was measured in the trunk, arm and leg in 26 SA and 26 Caucasian adults. We detected the pulses using Doppler or photoplethysmographic probes and calculated PWV as: PWV = (distance between the probes) / transit time.

Results: We found no significant PWV differences between the Caucasian and SA group, although the mean trunk PWV was higher in the SA group. Questionnaire analysis revealed that the SA population consumed significantly less fruit and vegetables than Caucasians. Systolic BP, pulse pressure, and trunk PWV were significantly higher in males.

Conclusion: Whilst others have found that older SAs have stiffer arteries, our study suggests that although this difference is not significant in young adults, it may become so with increasing age. Lifestyle differences between the groups suggest that perhaps more targeted health promotion is needed in the SA population. The observed gender differences may contribute to the increased risk of CVD seen in males.

P73

Coronary intraplaque hemorrhage evokes a novel atheroprotective macrophage phenotype

JJ Boyle[1], HA Harrington[2], E Piper[1], K Elderfield[3], J Stark[4], RC Landis[5], DO Haskard[1]

[1]Vascular Sciences, NHLI, Imperial College London, [2]Mathematics Dept, Imperial College London, [3]Imperial Healthcare NHS Trust, [4]Centre for Systems Biology, NHLI, Imperial College London, [5]University of the West Indies

Intraplaque hemorrhage accelerates atherosclerosis via oxidant stress and contributes to lesion development and destabilization. Normally, macrophages scavenge hemoglobin-haptoglobin (HbHp) complexes via CD163, and this process provokes the secretion of the anti-inflammatory atheroprotective cytokine interleukin (IL)-10. We therefore tested the hypothesis that HbHp complexes may drive monocyte differentiation to an atheroprotective phenotype.

Examination of the macrophage phenotype in hemorrhaged atherosclerotic plaques revealed a novel hemorrhage-associated macrophage population (HAmac), defined by high levels of CD163, but low levels of human leukocyte antigen-DR. HA-mac contained more iron, a pro-oxidant catalyst, but paradoxically had less oxidative injury, measured by 8-oxoguanosine content. Differentiating monocytes with HbHp complexes reproduced the CD163high human leukocyte antigen-DRlow HA-mac phenotype in vitro.

These in vitro HA-mac cells cleared Hb more quickly, and consistently showed less hydrogen peroxide release, highly reactive oxygen species and oxidant stress, and increased survival. Differentiation to HAmac was prevented by neutralizing IL-10 antibodies, indicating that IL-10 mediates an autocrine feedback mechanism in this system. Nonlinear dynamic modeling showed that an IL-10/CD163-positive feedback loop drove a discrete HA-mac lineage. Simulations further indicated an all-or-none switch to HA-mac at threshold levels of HbHp, and this conversion was experimentally verified. These data demonstrate the creation of a novel atheroprotective (HA-mac) macrophage subpopulation in response to intraplaque hemorrhage and raise the possibility that therapeutically reproducing this macrophage phenotype may be cardio-protective in cases of atherosclerosis.

P74

Eosinophilic Pleural Effusion with Charcot-Leyden crystals in a patient with invasive aspergillosis: a case report and review of the literature

C Smart[1], I Proctor[2], J Brown[2], G Kocjan[2]

[1]St George's Hospital, London, [2]University College Hospital, London

Eosinophilic pleural effusions (EPEs) are commonly associated with a number of pathological conditions, including pneumothorax, haemothorax, pleural malignancies, pulmonary embolism and drug reactions. There are also limited reports of EPEs being associated with bacterial, parasitic and fungal infections. Pathological processes involving eosinophils are occasionally associated with the formation of Charcot-Leyden crystals.

Here we report the case of a 30 year old woman who presented with asthmatic symptoms, gradually worsening shortness of breath and a peripheral eosinophilia. Investigations revealed a significant pleural effusion and a cavitating lesion in the upper lobe of her right lung. Cytological examination of the pleural fluid revealed a prominent EPE that was associated with numerous Charcot-Leyden crystals. The patient was treated by partial lobectomy and histological examination revealed invasive aspergillosis.

A review of the published literature identified a number of studies reporting an association between EPEs and a variety of different fungal infections. However, this is the first documented case of EPE arising in a patient with invasive aspergillosis. This case demonstrates the wide diagnostic differential of EPEs and nicely illustrates the associated phenomenon of Charcot-Leyden crystal formation.

P75

A retrospective review of primary squamous cell carcinomas of the lung – a review of 460 cases

M Deshmukh[1], P Goldstraw[2], E Lim[2], S Jordan[2], G Ladas[2], M Dusmet[2], S Popat[3], A Rice[1], A Nicholson[1]

[1]Department of Histopathology, Royal Brompton Hospital, London
[2]Department of Thoracic Surgery, Royal Brompton Hospital, London
[3]Department of Medical Oncology, Royal Marsden Hospital, London and National heart and Lung Institute, Imperial College, London

Although the frequency of squamous cell carcinoma (SQCC) of lung is said to be decreasing in relation to adenocarcinoma, little is known about how this relates to tumour location within the lung. The aim of this study was to assess resected squamous cell carcinomas, specifically in relation to central versus peripheral location.

A database of 460 surgical specimens received from January 1993 - April 2009 was retrospectively analysed to review parameters from the Royal College of Pathologists Minimum Dataset for lung cancers, adding tumour location. Central tumours were defined as those centred on main stem or lobar bronchi, or showing predominant growth within segmental airways.

Notable findings were: 1. Gradual decline in the incidence of resected SQCCs from 47 cases in 1993 to 24 cases in 2000 with the numbers remaining relatively steady thereafter; 2. Change in the ratio of central to peripheral location from 1.9 :1 in 1993-2001 to a ratio reversal of 0.7:1 in 2002-2009; and 3. Lower male:female ratio in peripheral versus central tumours, 2.4:1 versus 4.4:1. Synchronous pre-neoplastic disease and nodal involvement was commoner in central tumours and pleural involvement in peripheral tumours.

The decline in numbers of resectable primary lung squamous cell carcinomas reflects overall national figures, though our data show this is mainly in central tumours, with peripheral tumours becoming more common since 2000. This may be the result of change in the type and intake of cigarette smoke and/or divergent carcinogenetic pathways.

P76

Sudden cardiac death after emotional stress associated with physical restraint, assault and/or altercation

R Georgiou[1], SV Noronha[1], MN Sheppard[1-2]
[1]CRY Centre for Cardiac Pathology, Imperial College London,
[2]Department of Histopathology, Royal Brompton and Harefield NHS Foundation Trust

We retrospectively investigated 28 cases of sudden cardiac death (SCD) in relation to an emotionally stressful event such as physical restraint (n=11; 3 in police custody, 4 in mental health institution, 4 other), assault (9), altercation (4), fight (3) and chase with restraint (1). The aim of this study was to highlight deaths during emotional stress and the fact that the diagnoses were mainly of non-atherosclerotic origin (n=23, 82%). The mean age of SCD was 33.3+/-14.7, range 15-74 years. Almost half the cases (n=13, 46%) died with a morphologically normal heart at macroscopic and microscopic level, indicating the possibility of channelopathies such as Brugada syndrome, long/short QT syndrome and catecholaminergic polymorphic ventricular tachycardia. A proportion of these cases were mental health patients and were on antipsychotic medication (n=5). Cardiomyopathy was found in 4 patients with arrhythmogenic right ventricular cardiomyopathy (1), idiopathic left ventricular hypertrophy (2) and hypertrophic cardiomyopathy (1). Coronary artery pathology was also an important cause of death (n=8) including atherosclerosis (5), bridging of the left anterior descending coronary artery (2) and anomalous coronary artery (1). The remaining cases were neutrophilic myocarditis (1), heart transplant rejection (1) and complex congenital heart disease (1). This study emphasizes the importance of SCD with a morphologically normal heart, cardiomyopathy and coronary artery pathology during emotional stress. A thorough autopsy is essential in such cases where medicolegal issues are important.

P77

Cardiopulmonary causes of maternal deaths from 1994 to 2009 in the United Kingdom

K Ogo[1, 2], SV Noronha[2], E Shamil[3], S Lucas[3], MN Sheppard[1, 2]
[1]Department of Histopathology, Royal Brompton and Harefield NHS Foundation Trust Hospital, [2]CRY Centre for Cardiac Pathology, Imperial College London, [3]Department of Histopathology, St Thomas' Hospital

The UK maternal mortality rate has fluctuated between 11 and 14 per 100,000 in the period 1994 to 2005. The trend shows increasing number of deaths due to cardiopulmonary disease. The purpose of this study was to analyse maternal cardiopulmonary deaths in the UK. Eighty-one cases were identified retrospectively from our combined archives of autopsies and consultation cases from 1994 to 2009. Mean maternal age at death was 30 ± 7 years, range 15 to 49, with 26 (32%) aged 35 or over. Deaths occurred at pre-delivery (25), peri-delivery (14), and post-partum (42). The majority of deaths were due to cardiovascular cause (n=52, 64%), followed by pulmonary vascular disease (n=23, 28%) and thrombotic disease affecting heart and/or lungs (6). The leading causes of death were sudden cardiac death with morphologically normal heart (n=21, 26%), cardiomyopathy (n=13, 16%), pulmonary hypertension (n=13, 16%) and pulmonary embolic disease (n=10, 12%; pulmonary embolism (6), amniotic fluid embolism (4)). The dominant cardiomyopathies were dilated cardiomyopathy (5) and left ventricular hypertrophy (5). Other causes included coronary artery pathology (5), congenital heart disease (4), thrombotic thrombocytopaenic purpura (4), valvular disease (3) and aortic disease (3). Our analysis has shown a sharp decline in deaths due to congenital heart disease and pulmonary hypertension in the last four years. In contrast, deaths due to cardiac disease with normal heart and cardiomyopathy as well as pulmonary embolic disease have been rising progressively in the last 12 years.

P78

Inter- and intra-observer variation in a semi-quantitative scoring system for airway inflammation in paediatric bronchial biopsies

K Lawson[1], A Rice[1], S Seglani[2], M Tsartsali[2], E Lim[1], A Bush[1,2], AG Nicholson[1,2]
[1]Royal Brompton and Harefield NHS Foundation Trust, [2]NHLI Division, Imperial College

Quantitative assessment of pathology in endobronchial biopsies (EB) from children with airway diseases may influence therapeutic decisions. In order to assess their clinical value, pathologists will require a reproducible scoring system for individual parameters. Quantitative measurements and specific staining are expensive and time consuming. This study assesses the reproducibility of a semi-quantitative scoring system.

Two pathologists independently assessed EBs, on two separate occasions, from 20 children with airways disease for extent of goblet cell hyperplasia (GCH); basement membrane thickening (BMT), lymphocytes/plasma cells (LY/P) ; neutrophils (N); eosinophils (E) and seromucinous gland hyperplasia (SMGH), using a scale of normal, mild, moderate and severe, blinded to their previous scoring. Grading was based on the experience of one pathologist of >300 cases.

Intra-observer agreement was assessed using Cohen's Kappa, by pooling each component grade to form two series of measurements, with good agreement observed (coefficients of 0.61 and 0.62). Inter-observer agreement was assessed using Bland and Altman methods by calculating the mean of each of the two readings to form a composite score. Good agreement was also observed with the 12 point score with a mean difference (95% CI) of -1.29 (-0.65 to -1.93). SMGH was excluded as they were absent in 8 cases.

This quick, simple system is a practical and reproducible method to assess paediatric biopsies in tabular form.

P79

Analysis of specialist referrals in pulmonary pathology – do they represent good value for money?

C Rowan[1], R Florio[1], AC Wotherspoon[2], AG Nicholson[1-3]
[1]Department of Histopathology, Royal Brompton and Harefield NHS Foundation Trust, [2]Department of Histopathology, Royal Marsden Hospital, [3]NHLI Division, Imperial College

Referral for specialist opinion in pulmonary pathology is not unusual as many diseases are infrequently encountered. This has always been undertaken on an informal basis but with cross-charging now recommended by the College, this study was undertaken to determine the value of this practice, in terms of cost, time and definitive diagnosis.

50 consecutive referrals were retrospectively reviewed for: reason for referral, favoured diagnosis, final diagnosis by specialist, laboratory and pathologist costs and turnaround times. Clinical significance was viewed as major if referral probably avoided the need for further investigation or changed management.

Specialist opinion affirmed diagnoses in 26/50 cases, revised in 16/50 and favoured diagnoses in 8/50. 80% of reports were deemed to have major clinical significance. Average laboratory, pathologist and total costs were £68.83, £60.40 and £130.47 respectively, excluding lymphoma investigations and corporate costs. Average turnaround time was 5 days, excluding those over holidays, with 84% reported within 7 days. Prolonged reporting times were mainly due to referrers not sending blocks or co-reporting lymphoproliferative disease.

Specialist opinion affirmed the original diagnosis in 52% of cases, with 32% having diagnoses revised, most with likely major impact on management, avoiding the need for repeat intervention with resultant risk to patients and financial cost. The cost of referral, even when taking into account the inflationary increase of approximately 5% since 2008, appears to be offset by a high level of definitive diagnosis, negating further investigation.

P80

A case of hypertrophic cardiomyopathy with unusual phenotype and radiological features

C Rowan[1], MN Sheppard[1-2]
[1]Department of Histopathology, Royal Brompton and Harefield NHS Foundation Trust, [2]CRY Centre for Cardiac Pathology, Imperial College London

Hypertrophic cardiomyopathy (HOCM) is a common genetic cardiac disease with a heterogeneous natural history and morphology. We present an autopsy case of HOCM confirmed on histological examination of the heart but with the highly unusual phenotype of a restrictive cardiomyopathy.

This patient was diagnosed with HOCM on echocardiography at the age of 11. The clinical course was characterised by recurrent atrial arrhythmias which were refractory to all management options including multiple ablation procedures and MAZE surgery. In latter years there was a slow decline in function with eventual death from end-stage cardiac failure.

However, imaging including echocardiography and MRI scanning revealed normal ventricular wall thickness and contractile function with a filling pattern highly suggestive of a restrictive cardiomyopathy. In addition a band of bright late-enhancement was noted in the interventricular septum, suggestive of fibrosis in this area. Given the imaging appearances of a restrictive defect, this raised the question of whether the diagnosis was of HOCM or restrictive cardiomyopathy.

Post-mortem examination confirmed an enlarged heart with normal ventricular thickness and no macroscopic features of HOCM. However on histological examination along with extensive scarring of the atria and fibrosis of the interventricular septum, there was diffuse and severe biventricular myocyte disarray, diagnostic of HOCM.

This case illustrates an unusual clinical course of a common genetic cardiac disease along with important clinical-radiological-pathological correlation.

P81

Variation in both macroscopic and microscopic features of arrhythmogenic right ventricular cardiomyopathy (ARVC)

K Patel[1], SV Noronha[1], MN Sheppard[1-2]
[1]CRY Centre for Cardiac Pathology, Imperial College London,
[2]Department of Histopathology, Royal Brompton and Harefield NHS Foundation Trust

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a rare dominantly inherited cardiac disease characterised by ventricular arrhythmias and sudden cardiac death (SCD). We retrospectively identified 73 referrals of SCD with ARVC. Majority of cases were male (n=54) with a median age of 35 years. Nine cases had a family history of cardiac disease and 4 had received a clinical diagnosis of ARVC during life. SCD occurred during exertion (27), at home (22), community (13), hospital (4) and unknown (9). Where an opinion was provided by the referring pathologist (n=48) the macroscopic findings were ARVC (19), hypertrophy (9 LVH and 1 biventricular hypertrophy (BVH)), LV fibrosis (6), normal (6), dilated cardiomyopathy (5) and ischaemic heart disease (2). Of the 43 hearts we received, our macroscopic findings included ARVC (22), hypertrophy (6 LVH and RV fat, 3 LVH, 2 right ventricular hypertrophy, 1 BVH), LV fibrosis (5) and normal (4). Histologically our analysis identified the predominance of biventricular involvement (50), exclusive right ventricular involvement (18), and a smaller group with exclusive left ventricular involvement (5). ARVC is difficult to diagnose and is prone to misdiagnosis when relying on macroscopic findings alone, which can be variable. Our study shows that even with a specialist examination the spectrum of macroscopic findings in ARVC included hypertrophy, fibrosis and even no abnormal features. Therefore, a detailed histological analysis is essential to confirm the diagnosis. A correct diagnosis is important for screening family members as this is a genetically inherited disease.

P82

The determination of thromboembolus size using Doppler Ultrasound

MJ Martin[1], EML Chung[2], KV Ramnarine[1], AH Goodall[2], AR Naylor[1,2], DH Evans[1,2]
[1]University Hospitals of Leicester NHS Trust, [2]Department of Cardiovascular Sciences, University of Leicester, UK

Background: Thromboemboli entering the cerebral circulation are a common cause of stroke, especially following carotid surgery. Although emboli can be detected using Doppler Ultrasound, even when their composition is known there is no reliable method of determining the size of these emboli. Here we report the results of an in-vitro study investigating the relationship between embolus size and embolus signal intensity for fresh thrombus emboli and the comparison of these results to clinical data.
Method: Thrombus was formed simulating conditions associated with the formation of arterial thrombus formation in-vivo. 390 Doppler embolic signals were generated from different sizes of thrombus circulating in a pulsatile closed flow-circuit. Clinical comparison data included 751 embolic signals recorded during post-operative monitoring of a patient following carotid surgery.
Results: Our in-vitro data exhibited oscillations in signal intensity with increasing embolus size, which were consistent with theoretical predictions. Positive correlations between embolus diameter, signal intensity, and duration were observed (r>0.8, P≤0.01). Application of theoretical predictions to interpretation of clinical embolic signals was used to categorize thrombi into three size ranges
Conclusions: This study provides the first experimental evidence of the theoretical relationship between Doppler embolic signal intensity and thrombus size. Although embolic signals generally became more intense with increasing thrombus size, strong oscillations due to resonance effects were observed. Despite these non-linear effects, the relationship between Doppler signal intensity and thrombus size can be used to broadly estimate the size ranges of emboli observed during clinical monitoring.

P83

Audit of lung cancer as a second primary malignancy in women

N Pillay[1], A Merve[1], M Sheaff[1], S Baithun[1]
[1]Royal London Hospital

Background: Anecdotal observations by the authors suggest that an increasing number of women with lung cancer have had previous radiotherapy as part of their treatment for breast carcinoma. Studies investigating this association have extracted data from cancer registries whose criteria for determination of a second primary tumour are based on clinical parameters. Objective: Audit the incidence of a second primary malignancy benchmarking this to existing cancer registry norms. Methods: Ninety six women with lung cancer were identified from pathology records of the Royal London Hospital. The following parameters were examined: previous history of malignancy, history and site of previous radiotherapy, chemotherapy and tobacco use. In addition slides were re-examined to confirm the histological type and TTF-1 expression.
Results: A total of 15/96 women were identified to have developed TTF-1 positive lung cancer following previous treatment for breast carcinoma and other malignancies. 10/15 had a previous history of radiotherapy and 6/10 were also smokers. The histopathological spectrum was varied.
Conclusion: The incidence rate of lung tumours is congruent with the currently accepted standards for second primary malignancies in breast cancer survivors.The awareness of the risk of development of second malignancies may help inform patients and clinicians regarding quality of life and guiding therapeutic options especially for women who smoke.
This is the first pathology based study to document this using TTF-1 as a more objective tool of determining primary malignancy.

P84

On the concept of objectivity in digital image analysis

PJ Tadrous[1]
[1]Cellular Pathology Dept, Northwick Park Hospital, London

Background: The term 'objective' connotes a method that is based on facts and not influenced by personal opinions, perception or emotion. One often reads in the literature claims of objectivity for methods that use digital image analysis (IA). This paper takes a look at the literature on IA and asks whether such a claim is ever justified.
Methods: A literature review of a subset of papers pertaining to IA in biology or medicine is scrutinised to determine what evidence exists for objectivity in these methods.
Results: While IA may have many benefits (speed, idefatigability, systematicity, repeatability, standardisation, etc.), algorithms are devised and implemented by human beings who make subjective decisions at each stage of the algorithm design and implementation process.
Discussion: Most IA methods are repeatable deterministic processes with the very same subjective decisions of the programmer implemented slavishly each time the algorithm is run. Transferring decision making to a machine via an algorithm does not in itself convey objectivity, it merely hard-codes the programmers' subjectivity it into the process. Thus, while these repeatable automatic decisions may be based on facts (the pixel values of the input image) they are not free of personal opinions (the decisions of the programmer).
Conclusion: Repeatability and automaticity must not be confused with objectivity. A lack of objectivity, however, does not imply a lack of utility. Unless specific evidence of objectivity is provided, editors and reviewers should insist that any claims of objectivity be either removed or justified prior to allowing publication.

P85

Automated detection of AAFB in tissue sections: an application of segmentationless probabilistic quasineural image analysis

PJ Tadrous[1]
[1]Cellular Pathology Dept, Northwick Park Hospital, London

Background: Mycobacterial infection is a major cause of disease. In histology ZN-stained sections are used to detect the acid-alcohol fast bacilli (AAFB) but this is laborious and sparse bacilli are easily missed. Here a novel approach to image analysis is presented and demonstrated by its application to the automated detection of AAFB.

Methods: ZN-stained tissues from 2 patients (known to have AAFB) were used. Six datasets (of 500 consecutive images each) were captured from these slides (x60 dry objective). Two datasets were used in training the algorithm, the other 4 were used to test it. The algorithm uses multiderivative source potentiators and suppressors feeding into interconnected product nodes that result in a probability value for each image (the likelihood that it contains AAFB) and a spatial probability map showing the position of any bacillus. Unlike traditional image processing, there is no need for segmentation of foreground objects from the background.

Results: In each of the datasets, AAFB were presented in the top 10 ranking images (out of 500) despite AAFB being sparse (frequencies as low as 1 bacillus per 500 images) and many images containing potentially confounding carbol fuchsin-stained structures or debris. The algorithm detected AAFB, not just in the 'lying flat' position but also those that stand 'on end'.

Discussion: These results suggest the method has potential to save time and money in resource-poor health services. High volume throughput work is required to establish the performance of this method compared to a human observer.

P86

Digital stain separation for image analysis

PJ Tadrous[1]
[1]Cellular Pathology Dept, Northwick Park Hospital, London

Background: It is often desirable to perform image analysis on sections prepared for human interpretation e.g. nuclear chromatin analysis in archival samples or 3D reconstructions using sections requiring human delineation of structures of interest. Unfortunately such analyses are often more effective using stains with less complex contrast. Here an automated selective 'de-staining' method for digital images is presented.

Method: The image is separated into its RGB (red, green, blue) and HSI (hue, saturation, intensity) components. A mask of stained tissue is prepared by automatic p-tile thresholding. A single weighted inverted colour channel (the red channel is used for H&E, DAB immunoperoxidase and HVG) is then added to each of the 3 primary colour channels separately by an iterative algorithm that adjusts the weights to give minimum variance within the mask. The modified RGB channels are then recombined. This method is automatic requiring no pre-definition of stain colours.

Results: The method is demonstrated to 'de-stain' nuclei in H&E sections (and an image of isolated nuclei can be derived from this in some cases). An image of isolated DAB reaction product is produced with immunoperoxidase preparations counterstained with haematoxylin. Furthermore HVG is separated into its separate components with the same algorithm.

Discussion: Complex methods for colour separation exist (e.g. spectral pathology and colour deconvolution) but these require special apparatus or many input parameters. The present method, whilst not as generally applicable as the former, is automatic for commonly used stains and has scope for further development.

P87

Preservation of morphology and nucleic acid integrity in paraffin tissue – can we have our cake and eat it?

C Lloyd[1], S McKay[2], C Davies[1], G Stamp[2], G Thomas[2]
[1]Singleton Hospital, Swansea, [2]Imperial College London

Combination of the preservation of intact nucleic acids and preservation of morphology in paraffin embedded material is extremely difficult: solution to this problem could improve development of molecular testing in the routine pathology setting. We investigated the effect of fixation in "molecular friendly" fixatives on the quality of RNA on routinely collected human tissue. Tissue was supplied from the Wales Cancer Bank and material from the same operative specimen was either snap frozen or fixed in either neutral buffered formalin (NBF), FineFix, Z7 or Paxgene fixative prior to processing. Sections were cut from each block and RNA and DNA were extracted using an appropriate commercial kit and the RNA Integrity Number (RIN) analysed by Agilent Bioanalyser. DNA integrity was analysed using gel electrophoresis and multiplex PCR. The median RINs (range) were 7.3 (2.2-9) for frozen tissue, and 2.45 (1.8-2.6) for Z7, 2.3 (1.4-2.6) for FineFix and 2.2 (1.9-2.7) for NBF. Paxgene samples gave substantially higher RINs than any other fixative (4.75 range 1.6-6.4) and gave equivalent morphological when results to NBF samples, except for increased eosinophilia. DNA from frozen and from Paxgene samples showed high molecular weight DNA (10 kb), whereas DNA from blocks fixed in the other fixatives showed substantial degradation. The Paxgene fixed samples showed substantially improved preservation of intact nucleic acid without loss of morphology and may represent a substantial advance in combining molecular biology with pathomorphology.

P88

InView: the novel application of virtual microscopy for pathology and biomedical training

F Browne[1], J Diamond[1], A Askew[1], D McCleary[1], S Harvey[1], P Hamilton[2]
[1]i-Path Diagnostics Ltd, Belfast, N. Ireland, [2]Bioimaging, School of Biomedical Sciences, Queen's University, Belfast, N. Ireland

InView incorporates Virtual Microscopy technology to provide an innovative approach for education in diagnostic practice. Virtual slides and decision analysis are applied to support training and skill acquisition in pathology at undergraduate and postgraduate levels. The platform has been developed as an on-line training tool integrating digital slides, e-learning tools and video to provide virtual case material in pathology. The InView platform is currently used by the Thames Histopathology Training School (the largest in the UK) to train pathologists and biomedical students in diagnostic cytology and histology. Within the school there are 24 year 1 trainees and ~100 trainees in years 2 to 5 of the programme. Training is undertaken in 12 training centres located in London (Harrow to King's College), Kent, Surrey and Sussex.

Using InView, trainees can practice diagnostic techniques and learn to look for correct morphological features, on-line at any time using authenticated pathology cases.

The InView platform consists of a viewer whereby a digital slide for each case can be viewed, a clue panel which presents features to the user to help in diagnosis, a Bayesian Belief Network whereby the diagnosis of a trainee is compared to that of a pathologist and an instructional video detailing the diagnosis of the expert pathologist.

InView platform has been favourably received as an additional learning resource by trainees in the Thames Histopathology Training School reporting that it aided in bridging the gap between learning from text books and double headed reporting of real cases with consultants.

P89

Virtual microscopy for education and research in pathology

F Browne[1], J Diamond[1], A Askew[1], D McCleary[1], J James[2],
K Williamson[2], S Harvey[1], P Hamilton[2]
*[1]i-Path Diagnostics Ltd, Belfast, N. Ireland, [2]Bioimaging, School of
Biomedical Sciences, Queen's University, Belfast, N. Ireland*

Virtual Microscopy has opened up enormous opportunities in virtual training in
pathology, medicine and biomedical studies. Central to this is the ability to
easily author resources, so that they can be structured into a course and
integrated with other content for on-line delivery. i-Path have developed an on-
line authoring tool for virtual slide-based education called PathXL which is
being used across Europe to support education, training and skill acquisition in
pathology.

PathXL is a fully functional content management system allowing
management and hosting of virtual slides. Virtual slides can be organised under
specific topics, headings and chapters in a manner that is flexible to the needs
of the individual user. Associated text, images, videos, documents and power
point presentations can be integrated with virtual slides to support training in
histology and cytology. Additionally, the administrative interface allows the
management of users, slides and diagnostic assessments. PathXL has a
sophisticated web-based virtual slide viewer which facilitates on-line annotation
of slides.

PathXL is currently used in the University of Giessen to teach micro anatomy
to medical students. It is also used at QUB to deliver new undergraduate
courses in uropathology with annotated slide sets and MCQ assessments.
Questionnaires at the end of the course reported that annotated images enhanced
students ability to recognize morphological features and interaction with
PathXL increased their interest in pathology.

This presentation highlights the advantages of virtual microscopy and the
application of PathXL as a valuable teaching and assessment platform in
pathology.

P90

The Northern Ireland Virtual Tissue Archive

F Browne[1], A Lyons[2], A Askew[1], D McCleary[1], S Harvey[1],
P Hamilton[2]
*[1]i-Path Diagnostics Ltd, Belfast, N. Ireland, [2]Bioimaging, School of
Biomedical Sciences, Queen's University, Medical Biology Centre, Belfast*

The main barrier for progression in translational cancer research is the shortage
of high quality human samples that are linked to well defined clinical and
pathological information. Therefore, tissue banking is essential as it allows for
the extensive collection of well classified and appropriately stored tissue
samples. Presently, tissue banks exist as large volumes of microscopic glass
slides, which are prone to loss or breakage, and can be viewed in only one
location at a time. Virtual microscopy has recently revolutionised this practice
by providing a virtual image of the tissue section for storage and review.
The Northern Ireland Virtual Tissue Archive (NIVTA), has been established as
a key centralised resource to support tissue-based translational research where
tissues and samples are anonymously stored and linked with clinico-
pathological staging information. NIVTA digitally scans virtual slides using a
bank of scanners, stored centrally and managed using the PathXL virtual slide
platform. These can be instantly retrieved from storage using authorised access
and viewed on-line using a dedicated web-based PathXL viewer anywhere in
the world. To date, in excess of 25 Terabytes of virtual slide cases have been
stored. These digital slides facilitate trans-national collaboration on tissue based
research and enable external pathologists to review, score and digitally annotate
material from multiple locations, without physically sending slides. Digital
slides also provide a platform for the automated quantitative analysis of tissue
biomarkers, which will offer a more reliable and objective approach to tissue
biomarker search and translational research.

P91

Creating on-line assessments with PathXL for education, training and research

F Browne[1], A Askew[1], D McCleary[1], K Williamson[2], S Harvey[1],
P Hamilton[2]
*[1]i-Path Diagnostics Ltd, Belfast, Northern Ireland, [2]Bioimaging and
Bioinformatics Queen's University Belfast, Centre for Cancer Research
and Cell Biology,Belfast*

The application of Virtual Microscopy has been successfully employed to assist
in the areas of e-learning, training and research in pathology. PathXL is an on-
line portal which provides a comprehensive set of tools for the management and
authoring virtual slides. PathXL provides a Question and Answer (QA) wizard
from which on-line educational self-assessments; Tissue Microarray scoring
and External Quality Assurance programs are easily developed and published
through a web browser.

The QA wizard provides an intuitive interface from which diverse question
types including Multiple Choice Questions (MCQ) and free text can be set as
standalone questions or on user selected resources. The QA wizard is integrated
with a viewer where images can be viewed and annotated. All user created
content is fully editable and easily published on-line. Once published, tests can
be taken, scores centrally stored, statistics generated and feedback provided.
The pan-European competency test in pathology recently coordinated with
PathXL delivered tests to over 350 trainee pathologists across 26 countries in
Europe. Furthermore, the QA wizard has been employed to create MCQ
assessments for the 3rd year Medical course: Bladder Cancer Histopathology at
Queens University Belfast undertaken by 20 students. The MCQ assessments
allowed students to test their competency using virtual slides where key
features were annotated.

The novel platform provides a valuable tool for virtual slide-based education
and training by significantly enhancing the ease with which users can build
applications requiring questions, interaction, and on-line assessment.

P92

Tissue MicroArray: a centralised management framework

Y Wang[1], D McCleary[2], P Hamilton[1]
*[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast,
[2]i-Path Diagnostics Ltd*

Tissue Microarrays (TMA) are used extensively in tissue research for
biomarker evaluation and discovery. Virtual TMAs based on whole slide
imaging provide a strong platform for automated assessment of biomarkers
using computerised imaging. This study presents a centralised TMA virtual
slide management framework for TMA images and associated data with
ultrafast high performance image processing capabilities using computer
algorithms. Digitally scanned TMA virtual slides are archived on a central
server. An in-house developed cluster-based high performance computing
(HPC) platform also resides on the server featuring the use of 120 processor
cores and a centralised load balance approach for computer based TMA core
processing. The HPC platform is able to access a database storing TMA core
locations and features which complies with standardised TMA data exchange
specification. Users are able to submit TMA images via PathXL™ (i-Path
Diagnostics Ltd) and define appropriate algorithms for processing on the
cluster. Tests using an extensive series of non-small cell lung cancer TMAs and
a variety of nuclear and cytological biomarkers suggest that the proposed TMA
management framework is practical with a range of functionality and can be
easily extended. The central HPC cluster is ultra-fast. It can process 20GB of
TMA image data in less than 10 seconds, allowing each TMA core to be
processed in 0.05 seconds. This framework has the potential to significantly
enhance biomarker discover by providing a means for genuine high throughput
TMA analysis.

P93

Evolutionary image analysis using autonomous agents with swarm-based 'intelligence'

SS Cross[1], RF Harrison[2]
[1]*Academic Unit of Pathology, University of Sheffield,* [2]*Department of Automatic Control & Systems Engineering, University of Sheffield*

Conventional image analysis uses global thresholding / segmentation / decomposition techniques which often produce disappointing results when trying to isolate / identify features which are evident to human observers. We are developing a novel method of image examination using autonomous agents with swarm-based 'intelligence'. Variable numbers of individual agents are distributed randomly across an image and move around the image following the gradient of a chemical trail which has been left by other agents in proportion to their occupancy of patches on the image and to certain specified properties of the image at that point (e.g. the ratio of red to blue in a colour image). When parameters in the programme are optimised, agents can be produced which normally roam around the image randomly unless an area contains a specific feature which then causes dynamic aggregation of the agents at that point. Different types of agents can be used simultaneously to identify different features. This technique can be used to identify regions of interest in an image which can then be examined in detail by a human observer. It is thus potentially useful in vigilance tasks, such as cytological and mammographic screening, where human performance can degrade with fatigue and repetition.

Abstracts

Invited Speakers

Note: Presenter's name is shown in **bold**

S1

Inflammation and the cancer microenvironment

F Balkwill[1]
[1]*Institute of Cancer, Barts and the London School of Medicine and Dentistry*

The cells and mediators of inflammation form a major part of the epithelial tumour microenvironment. In some cancers, inflammatory conditions precede development of malignancy; in others, oncogenic change drives a tumour-promoting inflammatory milieu. Whatever its origin, this 'smouldering' inflammation aids proliferation and survival of malignant cells, stimulates angiogenesis and metastasis; subverts adaptive immunity, and alters response to hormones and chemotherapy. Cytokines are major mediators of communication between cells in the inflammatory tumour microenvironment and may be important therapeutic targets in cancer patients.

S2

Cancer genetics

I Tomlinson[1]
[1]*University of Oxford*

Recent progress in cancer genetics has centred on the discovery of common, low-penetrance predisposition alleles through genome-wide association studies. With particular reference to colorectal cancer, the findings from these studies are summarised. The studies have been successful in proving the common gene-common disease model for cancer, but have arguably been disappointing in terms of the effect sizes observed. Nevertheless, several insights have been gained, not least into the various different types of gene involved in cancer predisposition and, in some cases, consistent fucntional pathways being affected, albeit different genes and pathways in different cancer types. Although some researchers have criticised GWA studies, these analyses are in their early stages. Whilst alternative disease models, such as low-penetrance effects of rare variants, remain valid subjects for study, several different approaches are needed to discover as many of the genetic effects on cancer risk as possible.

S3

Evolution of the cancer genome

M Stratton[1]
[1]*The Wellcome Trust Sanger Institute, Cambridge*

All cancers carry somatically acquired changes in their genomes. Some, termed "driver" mutations, are causally implicated in cancer development. The remainder are "passengers", and bear the imprints of mutational processes operative during cancer development. Following the advent of second generation sequencing technologies the provision of whole cancer genome sequences has become a reality. These sequences will include comprehensive catalogues of somatic mutations, including point mutations, rearrangements and copy number changes and will provide insights into the evolutionary processes underlying the development of individual human cancers including the factors generating variation and the forces of selection. These insights will form the foundation of our understanding of cancer causation, prevention and treatment in the future.

S4

Viral and host contributions to HPV-associated squamous carcinogenesis: prospects for improved clinical management

N Coleman[1]
[1]*Medical Research Council Cancer Cell Unit, Hutchison/MRC Research Centre, Cambridge*

While the link between high risk human papillomavirus (HRHPV) infection and anogenital carcinoma is well established, viral and host mechanisms of neoplastic progression remain poorly understood, largely because of a paucity of detailed longitudinal data. We have studied HRHPV-associated squamous carcinogenesis in vitro using the unique W12 model system and validated our observations wherever possible using clinical samples. Recent progress includes:

1. We showed that HRHPV can be maintained for prolonged periods in keratinocytes as extra-chromosomal circular DNA (episomes). However, if episomes are cleared, cells emerge that contain integrated virus, associated with increased expression of the viral oncogenes E6/E7 and phenotypic progression from low-grade to high-grade pre-malignancy.

2. Viral integration can occur at any time during the period of episome maintenance and targets host genomic regions that are particularly accessible for insertion of foreign DNA. While the growth of most integrant-containing cells is predicted by viral oncogene levels, for some (~15%) growth rates are disproportionately high, indicating an additional contribution from host genes. We are currently investigating whether this represents HRHPV-induced insertional mutagenesis.

3. The selected integrant-containing cells are genomically unstable and develop in vitro invasiveness. The DNA copy number imbalances that develop are identical to those commonly seen in vivo. As they accumulate sequentially, it is relatively straightforward to identify candidate genes driving selection. To date, we have shown that genes showing the most significant expression increases following gain of chromosome 5p (a common imbalance in vivo) include oncostatin-M receptor, a targetable cell surface cytokine receptor, and Drosha, causing global changes in microRNA profiles.

S5

The role of the pathologist in sudden cardiac death

M Sheppard[1]

[1]Royal Brompton Hospital, [2]CRY Cardiac Pathology Laboratory, Imperial College, London

Sudden cardiac death is a significant cause of mortality mainly due to ischaemic heart disease. As a referral centre we receive a large number. Cardiac Risk in the Young (CRY) has funded a cardiac pathology laboratory for rapid diagnosis. Design: A total of 710 cases of sudden cardiac death in males (n=449) and females (n=261). These were diagnosed as morphologically normal heart, cardiomyopathies (CMP) including arrhythmogenic ventricular cardiomyopathy(ARVC), dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM) myocarditis (lymphocytic, giant cell, toxic or other) valvular defects, non-atheromatous coronary anomalies (abnormal anatomic origin or course, bridging, dissection, and vasculitis), congenital heart disease (CHD) and other causes.

Result: (50%) had a normal heart so channelopathies must be considered. 110 cases with CMP (16%), 108 with myocardial abnormality of unknown cause (15%) which comprised both myocardial hypertrophy (n=98) and fibrosis(n=10). Myocarditis in 41 cases (6%), valvular defects in 25 cases (3.5%), coronary abnormalities in 24 cases (3.4%), CHD in 10 cases (>1%) and other abnormalities in 35 cases (5%). Other included Sarcoidosis (n=15), Fabry s disease (n=3), Amyloidosis, Neoplasms (n=3, lipoma, mesothelioma) of AV node, microthrombosis (n=2), aneurysm of the membranous septum (n=1) and Libman-Sacks endocarditis.

The role of the pathologist is to examine the heart carefully, take 8-10 blocks at least for histology, make a correct diagnosis of the cardiac cause of death, take appropriate material for genetic analysis and provide a timely report for the coroner and family.

S6

Multi-Dimensional Fluorescence Imaging – for High Content and/or Label-Free Bioimaging

PMW French[1]

[1]Imperial College London

Fluorescence offers many opportunities for optical molecular imaging and can provide information beyond simply the localisation of fluorescent labels. At Imperial College London we are developing technology to analyse and image fluorescence radiation with respect to wavelength, polarisation and, particularly fluorescence lifetime, in order to maximise the information content. This talk will review our recent progress applying fluorescence lifetime imaging (FLIM) and multi-dimensional fluorescence imaging (MDFI) to tissue imaging and cell microscopy.

Applying FLIM to autofluorescence of biological tissue can provide label-free contrast for non-invasive diagnostic imaging, as we have demonstrated in various tissues, imaging cancer (skin, pancreas, colon, cervical tissue) osteoarthritis and atherosclerosis. FLIM and MDFI are also applicable to image intracellular structure and function for cell biology and drug discovery - in particular providing (quantitative) information concerning the local fluorophore environment and facilitating robust fluorescence resonant energy transfer (FRET) experiments. For high content analysis of complex samples including tissue sections, we have developed microscopes able to simultaneously resolve fluorescence with respect to fluorescence lifetime and emission/excitation spectra in three spatial dimensions. To increase throughput for drug discovery, we have demonstrated an automated high-speed optically-sectioned FLIM multiwell plate reader for fixed and live cells. In order to make more physiologically relevant measurements, we are developing tomographic FLIM instrumentation for application to embryos and small organisms and for clinical in vivo studies we have demonstrated FLIM endoscopy.

S7

Multi-dimensional ultrastructure

L Collinson[1]

[1]Cancer Research UK London Research Institute

Electron microscopy overcomes the resolution limitation inherent in light microscopy and can reveal the ultrastructure of cells and tissues. However, transmission electron microscopy (TEM) requires the sample to be cut into ultrathin sections which introduces sampling artifacts and masks interactions between cells and their environment in 3D space. In comparison, scanning electron microscopes (SEMs) can image larger samples but typically produce topographical or compositional information from only the surface layer.

Recent innovations have combined SEM imaging with *in situ* sectioning, leading to a paradigm shift in high resolution volume imaging of biological samples. In Focused Ion Beam/ Scanning Electron Microscopy (FIB/SEM) a slice of material is removed using a gallium ion beam, which is inherently destructive. In Serial Block Face/ Scanning Electron Microscopy (SBF/SEM) a slice of material is removed using a diamond knife in a modified ultramicrotome inside the SEM chamber. In both cases, the revealed surface is imaged using the scanning electron beam, and the cutting and imaging process is repeated sequentially to automatically collect a stack of high resolution images through the sample.

Data sets contain thousands of images that will revolutionise our understanding of cellular networks, developing organs and complex tissue organization. By correlating light microscopy and X-ray imaging with volume electron microscopy, we can locate areas of interest within cells, tissues and model organisms to give unprecedented three-dimensional structural and functional detail of transient biological processes *in vivo*.

S8

Pathologist-free microscopy?

R Bhargava[1], RK Reddy[1], FN Pounder[1], AK Kodali[1], M Schulmerich[1], MJ Walsh[1]

[1]University of Illinois at Urbana-Champaign

Histopathologic characterization has traditionally consisted of microscopy aided by simple stains and human interpretation. Conceptual ingenuity, a renaissance in biomedical research, technological developments and computation capabilities have all combined to drive an impressive growth in microscopic imaging and diagnostic assays in recent times. The pathologist's workload, however, has not been the focus of these technological advances as the new technology often provides new data but not definitive diagnostic information. More information and integration is required to augment microscopy in pathology from a visualization tool to an informative assay. This can be accomplished by chemical imaging. In chemical imaging, spectroscopy is used to measure both intrinsic molecular composition and structure of tissue – obviating the need for stains, dyes or special molecular probes. Computer algorithms then convert the rich data into information desired by pathologists. Here, we describe the development of mid-infrared spectroscopic imaging instrumentation, associated analytical methods and applications of this new technology. We especially focus on areas where the workload of the pathologist can be reduced, simple triage decisions can be made and currently unavailable capability can be added. We present recent results from prostate, breast and colon tissues to demonstrate how microscopy can include automated histopathologic recognition. The information can be used as stand-alone diagnostic information or as an adjunct to help pathology laboratories make rapid and efficient decisions. Last, we describe recent developments that will obviate the need for conventional microscopy procedures for a large fraction of samples in the routine analysis of biopsies.

S9

Developmental phenotyping: combining traditional pathology and cutting edge technologies

B Spencer-Dene[1-2], S Ding[1-2], G Stamp[1]
[1]Imperial College London, [2]The London Research Institute-CRUK

For decades scientists have studied genes that play defined roles during mammalian embryogenesis that have subsequently been shown to be involved in the aetiology and progression of diseases including cancer, and vice-versa. A good example of one such gene, is the IIIb isoform of the Fibroblast Growth Factor Receptor 2 (Fgfr2b), originally identified as an amplified gene product in gastric cancer. Recently the aberrant activation of Fgfr2b has been linked to other cancers most notably breast cancer, and also in several craniosynostosis syndromes. By studying the role of Fgfr2b during mammalian development using mouse models we established that it is fundamentally required for normal organogenesis, to the extent that mice null for this tyrosine receptor are perinatal lethal due to severe lung agenesis. In addition we have recently uncovered a potential role for Fgfr2b in gastroduodenal development and duodenal stenosis. Understanding the roles of Fgfr2b-mediated signalling during development will help to elucidate the mechanisms by which deregulation of these pathways leads to cancer, potentially helping to determine new therapeutic targets.This kind of specialized developmental phenotyping set within a dedicated mouse phenotyping unit, is an extremely useful complement to the more established veterinary pathologists and utilizes both traditional and cutting edge technologies to achieve that aim.

S10

Translational clinical trials

SE Pinder[1]
[1]King's College London, Research Breast Pathology, Guy's Hospital

Histopathologists have multiple roles in translational research within clinical trials and are involved in a variety of different aspects. At the simplest level, the provision of paraffin blocks of tissue is an increasing part of histopathology departments' role. The value of this reliable source of human tissues for biomedical research cannot be underestimated. Many national clinical trials require central review of pathology in order to confirm accuracy of diagnosis and entry criteria and some include a central reassessment of markers (such as ER or HER2 status of a breast cancer); histopathologists are also involved performing this essential quality control role. A smaller group of pathologists is more directly involved in translational clinical research, participating in academic aspects of the trial, actively undertaking molecular research, typically examining new putative prognostic or predictive biomarkers or searching for new targets for therapy. Finally, pathologists also play a vital role in the development and planning of clinical trials; their participation on scientific, steering and translational sub-committees is essential for the optimal design of protocols, particularly the logistics of obtaining tissue for translational research, as well as the preparation and quality of such specimens and the planned methodology and validity of assays.

Pathologists are thus involved in numerous aspects of translational clinical trials. Such translational clinical research is a resource-consuming activity, from the "routine" NHS histopathology laboratory to the more "academic" aspects. Adequate finances are required and it is vital that this should not be ignored, either by clinical colleagues and funding bodies.

S11

Fixed forever? Fixation and molecular techniques

G Thomas[1]
[1]Imperial College London

To reach the ultimate goal of personalised medicine will mean the marriage of morphology and molecular biology. Fixation in neutral buffered formalin, whilst being the gold standard for morphology and long term preservation, leads to significant problems for downstream molecular biology techniques. Some of these can be overcome by modifying the conditions of the assay, e.g. reducing amplimer size for RT-PCR, but for others, such as the array technologies, solutions are needed. Others, such as miRNA analysis, work perfectly well in FFPE tissue, and could quickly become useful clinical tests. There is an increasing interest in alternative methods of stabilisation of biomolecules, but many of these sacrifice the preservation of morphology. Maintaining archives of frozen tissue when their future use in molecular testing is unclear is not practical in the routine histopathology NHS setting. Therefore, strategies to identify methods for marker analysis that can be adapted for use on FFPE tissue, or methods of fixation which permit the use of one block of tissue for both morphology and molecular biology need to be developed. Pathologists need to become proactive in the use of human tissue for research on molecular markers of disease to ensure that these tests add value to routine pathological diagnosis, rather than become a more expensive way to replace it.

S12

Tissue specimens within the paradigm of 'fit for purpose'

S Hewitt[1]
[1]National Cancer Institute, National Institutes of Health, Bethesda, USA

The collection of human tissue for research engenders competing interests that challenge the biobanker. Tissue is routinely collected without reference or thought to what assays will be applied. Investigators are forced to use specimens of convenience, with little standardization or attention to detail, resulting in assays that fail to meet expectations. Apply the model of "Fit for Purpose" to tissue collection, where a specimen has a pre-ordained analytic future, assay development and validation is enhanced. This model pertains not only to how the biospecimen is collected, but to the assay that will be applied to the biospecimen. Applying this approach, tissues collected for biobanking can be collected based on specific analytes, and if required, specific assays. General analytes for which tissue might be collected are DNA, RNA or protein. Although different protocols may not be required for specimens collected for isolation of DNA, different approaches can be justified for specimens being utilized for global transcriptomic analysis versus PCR based assays of single transcripts. The goal is to match the biospecimen to the assays, ensuring quality results that accurately measure the target(s) of interest. Although this approach requires additional planning, it provides higher quality biospecimens to realize better biomarker assays.

S13

The future of cancer care

K. Sikora[1]
[1]CancerPartnersUK

There will be a dramatic increase in global cancer incidence over the next 20 years because of aging populations. Local forms of therapy will continue to improve achieving local destruction of cancers with greater certainty and fewer side effects. Minimally invasive surgery will reduce the need for routine organ resection without compromising survival. The application of sophisticated computer systems to radiotherapy planning will allow the precise shaping of beam delivery conforming exactly to the shape of the tumour. But the most promising advances on the horizon come from our rapidly increasing understanding of the molecular genetics of cancer. This will have considerable impact on prevention, screening, diagnosis and treatment and lead to a new golden age of drug discovery.

Because we know the precise targets of these new agents, there will be a revolution in how we prescribe cancer therapy. Instead of defining drugs for use empirically and relatively ineffectively for different types of cancer, we will identify a series of molecular lesions in tumour biopsies. Future patients will receive drugs that target these lesions directly. The human genome project provides a vast repository of comparative information about normal and malignant cells. The new therapies will be more selective, less toxic and be given for prolonged periods of time, in some cases for the rest of the patients life. This will lead to a radical overhaul of how we provide cancer care.

Presenter's Index