NEWCASTLE PATHOLOGY 2005

Hosted by the University of Newcastle-upon-Tyne and the Newcastle-upon-Tyne Hospitals NHS Trust

Venue
The University of Newcastle-upon-Tyne, Framlington Place, Newcastle-upon-Tyne, UK

There will also be Companion Meetings with the Association of Clinical Electron Microscopists, the Renal EQA, Liver EQA and UK NEQAS for Cellular Pathology Technique

5 – 8 July 2005

Third Joint Meeting of the British Division of the International Academy of Pathology and the Pathological Society of Great Britain and Ireland

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PROGRAMME ACKNOWLEDGEMENTS

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and the Pathological Society of Great Britain & Ireland

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TUESDAY 5 JULY

09.00–11.50 **Symposium:** *Forensic Aspects of Bones* (Lecture Theatre C)

09.00–17.00 **Slide Seminar Case Viewing** (MED DENE Room 519, 5th Floor, Cookson Building)

09.15–11.45 **Symposium:** *Pitfalls in Gynaecological Pathology* (David Shaw Lecture Theatre)

09.30–11.55 **Joint Symposium with The Royal Microscopical Society:** *Applications of Flow Cytometry and Confocal Microscopy in Pathology* (Lecture Theatre D)

10.15–11.15 **Coffee break** (Medical School Foyer) (sessions vary – please check detailed programme)

12.00–12.15 **Opening Address, Prof AD Burt, Newcastle** (David Shaw Lecture Theatre)

12.15–13.00 **Keynote Lecture, Prof RH Young, Boston:** *Perplexing patterns, perspicacious pathologists: reflections on the diversity of gonadal neoplasms and those who have described them* (David Shaw Lecture Theatre)

13.00–14.00 **Renal EQA** (Fenwick Room, Castle Leazes Halls of Residence)

13.00–14.00 **Lunch** (Dining Area, Castle Leazes Halls of Residence)

14.00–15.00 **Poster Presentations & Trade Exhibition** (Dining Area, Castle Leazes Halls of Residence)

15.00–17.00 **Oral Presentations** (David Shaw Lecture Theatre & Lecture Theatre C)

15.00–17.00 **Symposium:** *Research Ethics and Pathology – an Interactive Workshop on Practical Issues* (Lecture Theatre D)

16.00–16.30 **Tea break** (Medical School Foyer)

17.30–18.30 **Public Lecture, Professor Sir James Underwood, Royal College of Pathologists, London:** *The Public Face of Pathology* (Curtis Auditorium, Herschel Building)

19.00–20.00 **Civic Reception** (The Pandon Room, The Civic Centre)

WEDNESDAY 6 JULY

08.30–12.00 **Cancer Stem Cells Symposium** – Sponsored by Blackwell Publishing (David Shaw Lecture Theatre)

09.00–17.00 **Slide Seminar Case Viewing** (MED DENE Room 519, 5th Floor, Cookson Building)

09.00–12.00 **Liver Tumours Symposium** (Lecture Theatre C)

10.10–11.00 **Coffee break** (Medical School Foyer) (sessions vary – please check detailed programme)

12.15–13.00 **Pathological Society 26th CL Oakley Lecture, Dr KA Oien, Glasgow:** *Gene expression profiling and classification of adenocarcinoma by site of origin* (David Shaw Lecture Theatre)

13.00–14.00 **Liver EQA** (Fenwick Room, Castle Leazes Hall of Residence)

13.00–14.00 **Lunch** (Dining Area, Castle Leazes Halls of Residence)

14.00–15.00 **Poster Presentations & Trade Exhibition** (Dining Area, Castle Leazes Halls of Residence)

15.00–16.00 **Plenary Oral Presentations** (David Shaw Lecture Theatre)

16.00–16.30 **Tea break** (Medical School Foyer)

16.30–17.00 **Plenary Oral Presentations** (David Shaw Lecture Theatre)

17.15–18.00 **BDIAP 1st George Cunningham Lecture, Prof CW Elston, Nottingham:** *The modern management of the patient with breast cancer. A celebration of the role of the pathologist* (David Shaw Lecture Theatre)

18.45–20.30 **Recent Advances in GISTs** – Reception and Symposium sponsored by Novartis Pharmaceuticals (Alnwick Suite, St James's Park)

THURSDAY 7 JULY

09.00–12.00 **Oral Presentations** (David Shaw Lecture Theatre & Lecture Theatre D)

09.00–12.00 **Slide Seminar:** *Transplant Pathology* (Lecture Theatre C)

09.40–17.00 **Companion Meeting: Association of Clinical Electron Microscopists** (Lecture Theatre A)

09.00–15.00 **Companion Meeting: UK NEQAS for Cellular Pathology Technique** (Fenwick and MGM Rooms, Castle Leazes Halls of Residence)

10.30–11.30 **Coffee break** (Medical School Foyer / Castle Leazes for UK NEQAS Meeting) (sessions vary – please check detailed programme)

12.00–13.00 **Pathological Society Annual Business Meeting** (David Shaw Lecture Theatre)

13.00–14.00 **Lunch** (Dining Area, Castle Leazes Halls of Residence)

14.00–15.00 **Poster Presentations and Trade Exhibition** (Dining Area, Castle Leazes Halls of Residence)

15.00–17.00 **Symposium:** *Approaches to Macroscopic Pathology and Minimum Data Sets* (David Shaw Lecture Theatre)

15.45 **Tea break** (Medical School Foyer)

17.15–18.00 **Pathological Society's 3rd Doniach Lecture, Prof Sir Dillwyn Williams, Cambridge:** *Thyroid Carcinogenesis Genotype-Phenotype correlation and The Effects of Radiation* (David Shaw Lecture Theatre)

18.15 prompt! **Buses leave from Medical School for the Conference Dinner**

19.30–23.00 **Conference Dinner** (Alnwick Castle, Northumberland)

FRIDAY 8 JULY

09.00–12.00 **Symposium:** *Controversies in Cellular Pathology* (David Shaw Lecture Theatre)

10.40–11.00 **Coffee break** (Medical School Foyer)

12.00–13.00 **Take-away Lunch** (Medical School Foyer)

ORAL COMMUNICATIONS

Sessions will be held as follows:

Tuesday 5 July	15.00–17.00	(David Shaw Lecture Theatre and Lecture Theatre C)
Thursday 7 July	09.00–12.00	(David Shaw Lecture Theatre and Lecture Theatre D)

Note to presenters: *Speakers are reminded that no communication may exceed the time allocated on the programme without the consent of the meeting, obtained through the Chairman.*

PLENARY ORAL SESSION

The six highest-ranked submitted oral abstracts will be presented on:

Wednesday 6 July 15.00–17.00 (David Shaw Lecture Theatre)

A prize for the best presentation, donated jointly by the *Journal of Pathology* and *Histopathology* will be presented at the Conference Dinner.

POSTERS / VIEWING

Posters will be displayed on Tuesday 5, Wednesday 6 and Thursday 7 July (Dining Area, Castle Leazes Halls of Residence). Poster viewing will be from 14.00–15.00 on all days. **Poster round chairmen will circulate during these times.**

Note to presenters: *Ideally, posters should be in place by 09.00 hrs on Tuesday 5 July and removed by 18.00 hrs on Thursday 7 July. At least one of the contributors must be in attendance during the viewing period, as indicated in the programme synopsis.*
The Sir Alastair Currie Prize and second and third poster prizes will be presented at the Conference Dinner.

SYMPOSIA

Tuesday 5 July

09.00–11.50	*Forensic Aspects of Bones* (Lecture Theatre C)
09.15–11.45	*Pitfalls in Gynaecological Pathology* (David Shaw Lecture Theatre)
09.30–11.55	Joint Symposium with the Royal Microscopical Society: *Applications of Flow Cytometry and Confocal Microscopy in Pathology* (Lecture Theatre D)
15.00-17.00	*Research Ethics and Pathology: An Interactive Workshop on Practical Issues* (Lecture Theatre D)

Wednesday 6 July

08.30-12.00	*Cancer Stem Cells* (David Shaw Lecture Theatre)
09.00–12.00	*Liver Tumours* (Lecture Theatre C)
18.45–20.30	*Recent Advances in GISTs* (Alnwick Suite, St James Park)

Thursday 7 July

15.00-17.00	*Approaches to Macroscopic Pathology and Minimum Data Sets* (David Shaw Lecture Theatre)

Friday 8 July

09.00-12.00	*Controversies in Cellular Pathology* (David Shaw Lecture Theatre)

Details of topics are listed in the Detailed Programme (see page 11) along with chairmen (correct as at time of going to press).

SLIDE COMPETITION & SEMINAR: *Transplant Pathology*

Competition

There will be a slide competition using digital slide images, which will be available for viewing on Tuesday 5 July and Wednesday 6 July (MED DENE Room 519, 5th Floor, Cookson Building). A joint BDIAP/Pathological Society prize will be awarded at the Conference Dinner.

Seminar

The follow-up seminar will be held on Thursday 7 July from 09.00–12.00 (Lecture Theatre C).

KEYNOTE AND NAMED LECTURES

(David Shaw Lecture Theatre)

Tuesday 5 July

12.15–13.00 **Keynote Lecture, Prof RH Young, Boston:** *Perplexing patterns, perspicacious pathologists: reflections on the diversity of gonadal neoplasms and those who have described them.*

Wednesday 6 July

12.15–13.00 **CL Oakley Lecture, Dr KA Oien, Glasgow:** *Gene expression profiling and classification of adenocarcinoma by site of origin.*

17.15–18.00 **George Cunningham Lecture, Prof CW Elston, Nottingham:** *The modern management of the patient with breast cancer. A celebration of the role of the pathologist.*

Thursday 7 July

17.15–18.00 **Doniach Lecture, Prof Sir Dillwyn Williams, Cambridge:** *Thyroid carcinogenesis, genotype-phenotype correlation and the effects of radiation.*

PUBLIC LECTURE

(Curtis Auditorium, Herschel Building, University of Newcastle-upon-Tyne)

Tuesday 5 July

17.30–18.30 **Prof Sir James Underwood, London:** *The Public Face of Pathology.*

TRADE EXHIBITION

(The Dining Area, Castle Leazes Hall of Residence)

Delegates are encouraged to visit the Trade Exhibition and are requested to support the companies represented there. There will be a Trade Exhibition Prize Quiz.

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:

For each full day: 7 points

For each half day: 3 points

Delegates who are eligible for CPD points should collect their certificates at the Registration Desk before leaving the Meeting.

This Meeting has also been approved by the **Institute of Biomedical Science** for CPD accreditation: 10 credits per day, 5 credits for Friday (Code: CP038N05).

REGISTRATION

Registration is **only** available via: **http://pathsoc.conference-services.net/directory.asp**

FEES

Fees include all refreshments and lunch.

BDIAP and Pathological Society Members

Up to and including 6 June 2005

£210 for the whole meeting, or £85 per day (or part day)

After 6 June 2005

£315 for the whole meeting, or £105 per day (or part day)

Non-Members

Up to and including 6 June 2005

£265 for the whole meeting, or £105 per day (or part day)

After 6 June 2005

£365 for the whole meeting, or £125 per day (or part day)

Special Categories

Individuals from the following categories qualify for nominal fees of £16 per day (or part day):

1. Junior Technicians*
2. Postgraduate Students*
3. Residents*
4. Trainees (SHO and SpR)*
5. Senior and Honorary Pathological Society Members
6. Senior and Honorary BDIAP Members
7. Undergraduates.*
8. UK NEQAS and Royal Microscopical Society delegates
9. BMS staff

* Delegates from categories 1, 2, 3, 4 and 7 must provide identification documents 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 and should be sent to: julie@pathsoc.org.uk or faxed to: +44 (0)20 7976 1267.

Conference Dinner

The charge for the Conference Dinner is £40 per person.

DELEGATE ENROLMENT

Enrolment will take place from 08.00 hrs on Tuesday 5 July (Foyer of the Medical School, Framlington Place).

ENQUIRIES

Enquiries before the Meeting regarding administration should be directed to:

British Division of the IAP
PO Box 73, Westbury-on-Trym, Bristol BS9 1RY
Tel: +44(0)117 907 7940
Fax: +44(0)117 907 7941
E-mail: bdiap@blueyonder.co.uk

OR:

Pathological Society of Great Britain and Ireland
2 Carlton House Terrace, London, SW1Y 5AF
Tel: +44 (0)20 7976 1260
Fax: +44 (0)20 7976 1267
Email: admin@pathsoc.org.uk

PRESENTATIONS

Presentation Checking & Preview

Presentation checking and preview will be available in Lecture Theatre E.

Oral Presentations / Lectures

To assist with the smooth running of the meeting, presenters are requested to send their presentations in *PowerPoint*® to the following contact in advance of the Meeting to arrive no later than Friday 24 June:

E-mail a compressed file
to: emma.reynolds@ncl.ac.uk

Presenters should ensure that they also bring a back-up copy to the meeting

OR

Send a CD
to: Emma J. Reynolds, PA to the Dean of Clinical Medicine,
Faculty of Medical Sciences, University of Newcastle upon Tyne,
Room 2.15, Department of Pathology,
Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4LP

Please label with name of presenter, abstract number and day of presentation

Slide Seminar

PCs for Slide Seminar Viewing will be located in MED DENE Room 519, 5th Floor, Cookson Building.

MESSAGES

During the Meeting, messages for delegates may be left at the following telephone number:

+44 (0)191 222 7816

There will also be a message board located beside the Registration Desk.

Delegates will be able to access Web Mail using PCs in the MED DENE Room 519.

REFRESHMENTS

Coffee and tea will be served in the Medical School Foyer and lunch will be served in The Dining Area, Castle Leazes Halls of Residence, where the Trade Exhibiton and Posters will be found. Shuttle buses will be available to transport delegates from the Medical School to Castle Leazes.

BADGES

Delegates are requested to wear their badges at all times.

TRAVEL

Newcastle is located in the north-east of England and within easy reach of other major UK cities of Edinburgh, York and Leeds. It is also easily accessible from all parts of the UK, Europe and beyond.

The Medical School is adjacent to the Royal Victoria Infirmary. Castle Leazes (Halls of Residence) is opposite the Medical School and is in easy walking distance. However, delegates staying at Castle Leazes are advised to take a taxi from the Airport.

Flights to Newcastle International Airport

There are direct flights to Newcastle International Airport from 15 UK destinations (including Bristol, Belfast, Cardiff and Aberdeen) and 22 European destinations (including Barcelona, Paris, Prague, Rome and Düsseldorf). There are excellent international connections via London airports and Amsterdam Schiphol. Newcastle International Airport is just 22 minutes by Metro from the City Centre.

The Metro

The City Centre is 20 minutes from the airport by Metro. Haymarket Station is closest to the Medical School and Castle Leazes.

Trains

Those arriving by train are advised to take a taxi from the Newcastle Central Station to Castle Leazes or local hotels.

Parking

There is no parking available within the Medical School grounds. The nearest car park (Newcastle City) is situated on Claremont Road. Car parking facilities are available at some of the Conference hotels and at Castle Leazes Halls of Residence.

Local Places of Interest

For local places of interest and activities please see:
www.pathology.visitnewcastlegateshead.com

MAPS

Detailed maps are located on the inside front and back covers of this Programme.

ACCOMMODATION

Accommodation had been arranged for delegates at hotels and Halls of Residence (Castle Leazes). All bookings should be made via the official accommodation booking website at
www.pathology.visitnewcastlegateshead.com.

The deadline for accommodation bookings is: **Monday 6 June 2005**. After this date rates and availability cannot be guaranteed. Hotels in Newcastle/Gateshead tend to get extremely busy, particularly on Tuesday and Wednesday nights, so you are advised to make your booking well in advance.

SMOKING

Smoking is prohibited at all meetings and social events except in the designated areas.

DISCLAIMER

The British Division of the International Academy of Pathology and the Pathological Society of Great Britain & Ireland cannot be held responsible for any injury or loss sustained during the Meeting.

SOCIAL ACTIVITIES

Tuesday 5 July

Civic Reception (Civic Centre, Newcastle-upon-Tyne).
Please reserve your ticket when registering – places are limited.

Thursday 7 July

Conference Dinner (Alnwick Castle, Northumberland).
Please reserve your ticket (cost £40) when registering – places are limited.

PROGRAMME – *Important Reminder*

Members of the BDIAP and the Pathological Society attending the Meeting **must bring this Programme with them** as only a limited number of copies will be available at the Meeting.

FUTURE MEETINGS

British Division of the IAP

2005 (25–26 November) London
Symposium on Intestinal Disease

2006 (12–13 May) Utrecht
Symposium on Soft Tissue & Bone Pathology

2006 (17–22 September) Montreal
IAP International Congress

2007 (Spring) Dublin
Symposium on Gynaecological Pathology

2008 (Autumn) Athens
IAP International Congress

Pathological Society of Great Britain & Ireland

2006 (4–6 January) Cambridge
Winter Meeting

2006 (4–7 July) Manchester
Centenary Meeting

2007 (3–5 January) London
Winter Meeting

2008 (January)
Winter Meeting

2008 (July)
Summer Meeting

2009 (January)
Winter Meeting

Joint Meetings (of the British Division of the IAP and the Pathological Society of Great Britain & Ireland)
2007 (3–6 July) Glasgow
Glasgow Pathology 2007 (4th Joint Meeting)

TUESDAY 5 JULY

▶ PARALLEL ◀ **09.00–11.50** **Lecture Theatre C**

SYMPOSIUM: *Forensic Aspects of Bones*
Chair: Dr PN Cooper, Forensic Unit, Royal Victoria Infirmary, Newcastle-upon-Tyne

09.00–09.35 ***Forensic anthropology***
Dr J Roberts, University of Glasgow

09.35–10.10 **[229]** ***The interpretation of fractures at autopsy***
Dr J Clark, University of Glasgow

10.10–10.45 ***Identification and ageing of fractures***
Prof AJ Malcolm, The Royal Shrewsbury Hospital and Keele University

10.45–11.15 **Coffee** (Medical School Foyer)

11.15–11.50 ***Cleaning and interpreting marks on bones***
Prof GN Rutty, University of Leicester and Leicester Royal Infirmary

▶ PARALLEL ◀ **09.15–11.45** **David Shaw Lecture Theatre**

SYMPOSIUM: *Pitfalls in Gynaecological Pathology*
Chair: Dr LJR Brown, Leicester Royal Infirmary
Dr JN Bulmer, University of Newcastle-upon-Tyne

09.15–09.45 **[230]** ***Morphologically bland vulvovaginal mesenchymal lesions: an update***
Prof WG McCluggage, Royal Group of Hospitals, Belfast

09.45–10.15 **[231]** ***Diagnostic pitfalls in smooth muscle and stromal tumours of the uterus***
Dr TP Rollason, Birmingham Women's Hospital, Birmingham

10.15–10.45 **Coffee** (Medical School Foyer)

10.45–11.15 **[232]** ***Diagnostic difficulties in gestational trophoblastic disease***
Prof M Wells, University of Sheffield, Medical School

11.15–11.45 **[233]** ***Problems in endometrial hyperplasia***
Dr CH Buckley, Manchester

▶ PARALLEL ◀ **09.30–11.55** **Lecture Theatre D**

JOINT SYMPOSIUM WITH THE ROYAL MICROSCOPICAL SOCIETY:
Applications of Flow Cytometry and Confocal Microscopy in Pathology
Chair: Prof MR Alison, Centre for Diabetes and Metabolic Medicine, Institute for Cell and Molecular Science, Queen Mary University of London

09.30–10.00 **Overview: *Confocal microscopy applications in pathology***
Professor R Poulsom, Histopathology Unit, Cancer Research UK, London

10.00–10.30 **Overview: *Flow cytometry applications in pathology***
Dr D Davies, FACS Laboratory, London Research Institute, Cancer Research UK

10.30–10.55 **Coffee** (Medical School Foyer)

10.55–11.15 ***Go with the flow! Applications in a regional centre***
Dr B Shenton, Flow Cytometry Facility, University of Newcastle-upon-Tyne

11.15–11.35 ***Imaging Langerhans cells in human epidermal sheets***
Dr M Collin, School of Clinical and Laboratory Sciences, University of Newcastle-upon-Tyne

11.35–11.55 ***Combining flow cytometry and confocal microscopy to study T cell extravasation***
Prof J Kirby, Applied Immunobiology and Transplantation Research Group, University of Newcastle-upon-Tyne

TUESDAY 5 JULY *continued*

▶ PLENARY ◀ **12.00–13.00** **David Shaw Lecture Theatre**

12.00–12.15 **OPENING ADDRESS**
Prof AD Burt, University of Newcastle-upon-Tyne

12.15–13.00 **KEYNOTE LECTURE:**
[248] ***Perplexing patterns, perspicacious pathologists: reflections on the diversity of gonadal neoplasms and those who have described them***
Chair: Prof AD Burt, Newcastle
Prof RH Young, Massachusetts General Hospital, Harvard Medical School, Boston, USA

▶ PARALLEL ◀ **13.00–14.00** **Fenwick Room · Castle Leazes Halls of Residence**

13.00–14.00 **COMPANION MEETING: Renal Pathology EQA**
Chair: Prof PN Furness, University of Leicester

13.00–14.00 **Dining Area · Castle Leazes Halls of Residence**

13.00–14.00 **LUNCH AND TRADE EXHIBITION**

▶ PLENARY ◀ **14.00–15.00** **Dining Area · Castle Leazes Halls of Residence**

POSTER VIEWING AND TRADE EXHIBITION
CATEGORIES
Cardiovascular/Pulmonary **[45-51]**
Genitourinary/Renal **[52–68]**
Gynaecological **[69-86 and 88–96] ***
Osteoarticular/Soft Tissue **[218]**
***** Poster number 87 (Gynaecological) will be displayed at the Association of Clinical Electronic Microscopists Meeting on Thursday 7 July.

▶ PARALLEL ◀ **15.00–17.00** **Lecture Theatre D**

SYMPOSIUM: ***Research Ethics and Pathology: an interactive workshop on practical issues***
Chair: Prof PA Hall, Queen's University Belfast
Dr AJ Howat, Burnley General Hospital, Burnley

15.00–15.40 **[234]** ***Research ethics: principles and current practice in the UK***
Dr SEH Russell, Queen's University Belfast

15.40–16.20 **[235]** ***The Human Tissue Act: what it will mean for you***
Prof PN Furness, University of Leicester

16.20–17.00 ***The practicalities of biobanking and using banked samples***
Dr BJ Clark, National Cancer Tissue Resource, Cancer Research UK, London

▶ PARALLEL ◀ **15.00–16.00** **David Shaw Lecture Theatre**

ORAL COMMUNICATIONS: ***Gynaecological, Autopsy and Forensic***
Chair: Prof P Domizio, St Bartholomew's Hospital, London
Prof CS Herrington, University of St Andrews, Scotland

15.00 **[1]** ***Angiogenesis markers in ovarian cancer act as prognostic markers but not indicators of chemotherapy response***
{P} S O'Toole, BL Sheppard, JJ O'Leary, O Sheils, T D'arcy, N Gleeson, E McGuinness, A Laois, J Bonnar

15.15 **[2]** ***Histomorphometric characterisation of shared and non-shared cotyledonary villus territories in monochorionic placentae in relation to pregnancy complications***
{P} LY Wee, NJ Sebire, M Sullivan, NM Fisk

15.30 **[3]** ***An immunohistochemical comparison between low grade and high grade ovarian serous carcinomas***
{P} CJ O'Neill, MT Deavers, A Malpica, H Foster, WG McCluggage

15.45 **[4]** ***The quality of autopsy reports 1994-2004 – do we meet the RCPath standards?***
{P} R J Delaney, I S D Roberts

▶ PARALLEL ◀ 15.00–16.00 Lecture Theatre C

ORAL COMMUNICATIONS: ***Genitourinary/Renal***
Chair: Prof S Fleming, Medical School, University of Dundee
Dr S Wells, Royal Bolton Hospital

15.00 **[7]** ***Immunohistochemistry for C4d in renal allograft biopsies: which method should be used?***
{P} ISD Roberts, LJ McWilliam, A McEwen, D Koo, I Quiroga, J Procter, MCNM Barnardo, M Sutton, L Cerundolo, DR Davies, PJ Friend, PJ Morris, SV Fuggle

15.15 **[8]** ***Bone marrow-derived cells contribute to a small extent to regeneration of renal parenchyma after injury***
{P} EI Prodromidi, D Turnberg, CA Roufosse, R Jeffery, CD Pusey, R Poulsom, HT Cook

15.30 **[9]** ***Antibody mediated rejection: diagnosis and correlation with renal graft outcome***
{P} LJ McWilliam, A McEwen, JE Worthington, S Martin, ML Picton

15.45 **[10]** ***Bone marrow-derived cells and mesenchymal-epithelial transition in kidney fibrosis***
{P} CA Roufosse, G Bou-Gharios, E Prodromidi, R Jeffery, T Hunt, C Alexakis, S Khan, T Cook, R Poulsom

16.00–16.30 Medical School Foyer

TEA

▶ PARALLEL ◀ 16.30–17.00 David Shaw Lecture Theatre

ORAL COMMUNICATIONS: ***Autopsy and Forensic***
Chair: Prof P Domizio, St Bartholomew's Hospital, London
Prof CS Herrington, University of St Andrews, Scotland

16.30 **[5]** ***Post mortem bacteriology: a re-evaluation***
{P} J A Morris, L M Harrison, S M Partridge

16.45 **[6]** ***Should facial protection be worn during post-mortem examination?***
{P} M.R Rao, C Phelan

▶ PARALLEL ◀ 16.30–17.00 Lecture Theatre C

ORAL COMMUNICATIONS: ***Neonatal/Paediatric and Cardiovascular/Pulmonary***
Chair: Prof S Fleming, Ninewells Hospital and Medical School, Dundee
Dr S Wells, Royal Bolton Hospital

16.30 **[11]** ***Comparative expression analysis of Pax3 and Pax7 during myogenesis in the mouse embryo***
{P} D. Horst, C. Sergi, G. Mikuz, H. Juergens, E. Vorobyov

16.45 **[12]** ***Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH). Clinicopathological correlation***
{P} S J Davies, J R Gosney, A U Wells, D M Hansell, R M du Bois, M M Burke, M N Sheppard, A G Nicholson

TUESDAY 5 JULY *continued*

▶ PLENARY ◀ **17.30–18.30** **Curtis Auditorium · Herschel Building**

PUBLIC LECTURE

[236] ***The Public Face of Pathology***
Prof Sir James Underwood, President, The Royal College of Pathologists, London

Chair: Prof OFW James, University of Newcastle-upon-Tyne

19.00–20.00 **Pandon Room · Civic Centre**

CIVIC RECEPTION
Hosted by the Lord Mayor and Lady Mayoress
(Councillor David Slesenger and Councillor Jackie Slesenger)

WEDNESDAY 6 JULY

▶ PARALLEL ◀ **08.30–12.00** **David Shaw Lecture Theatre**

SYMPOSIUM: ***Cancer Stem Cells*** – Sponsored by Blackwell Publishing
Chair: Dr C Sarraf, University of Westminster, London
Prof NA Wright, St Bartholomew's and The Royal London School of Medicine and Dentistry

08.30–08.55 ***Stem cells in the development of colon cancer: bridging the molecular gap***
Prof NA Wright, St Bartholomew's and The Royal London School of Medicine and Dentistry

08.55–09.20 ***Cancer stem cells: lessons from leukaemia***
Dr D Bonnet, Haematopoietic Stem Cell Laboratory, Cancer Research UK, London

09.20–09.45 ***Isolation and characterisation of human mammary stem cells***
Dr R Clark, Breast Biology Group, Cancer Research UK, University of Manchester

09.45–10.10 ***Stem cell patterns in cell lines derived from epithelial cancers***
Prof IC Mackenzie, Centre for Cutaneous Biology, Institute for Cell and Molecular Science, Queen Mary University of London

10.10–10.40 **Coffee** (Medical School Foyer)

10.40–11.05 ***Hepatocarcinogenesis: are liver stem cells involved?***
Prof MR Alison, Centre for Diabetes and Metabolic Medicine, Institute for Cell and Molecular Science, Queen Mary University of London

11.05–11.30 ***Cancer stem cells in the mammalian CNS***
Prof GJ Pilkington, Cellular and Molecular Neuro-oncology Research Group, University of Portsmouth

11.30–11.55 ***Epithelial stem cells in human prostate growth and disease***
Dr D Hudson, Prostate Stem Cell Laboratory, Institute of Cancer Research, MUUCRC, Surrey

▶ PARALLEL ◀ **09.00–12.00** **Lecture Theatre C**

SYMPOSIUM: ***Liver Tumours***
Chair: Dr JI Wyatt, United Leeds Teaching Hospitals NHS Trust, Leeds
Prof AD Burt, University of Newcastle-upon-Tyne

09.00–09.30 **[237]** ***Approaches to liver tumour resection***
Dr JI Wyatt, United Leeds Teaching Hospitals NHS Trust

09.30–10.00 ***Hepatocellular carcinoma and precursor lesions***
Dr A Quaglia, King's College Hospital, London

10.00–10.30 ***Cholangiocarcinoma and mixed tumours***
Prof SG Hubscher, University of Birmingham

10.30–11.00 **Coffee** (Medical School Foyer)

11.00–11.30 **[238]** ***Vascular tumours***
Prof AD Burt, University of Newcastle-upon-Tyne

11.30–12.00 **[239]** ***Paediatric liver tumours***
Prof A Zimmerman, University of Berne, Switzerland

▶ PLENARY ◀ **12.15–13.00** **David Shaw Lecture Theatre**

PATHOLOGICAL SOCIETY OF GREAT BRITAIN & IRELAND'S
26TH CL OAKLEY LECTURE
[240] ***Gene expression profiling and classification of adenocarcinoma by site of origin***
Dr KA Oien, Cancer Research UK Beatson Laboratories and University of Glasgow
Chair: Professor M Pignatelli, University of Bristol and Bristol Royal Infirmary

WEDNESDAY 6 JULY *continued*

▶ PARALLEL ◀ **13.00–14.00** **Fenwick Room · Castle Leazes Halls of Residence**

COMPANION MEETING: ***Liver EQA***
Chair: Dr JI Wyatt, United Leeds Teaching Hospitals NHS Trust

13.00–14.00 **Dining Area · Castle Leazes Halls of Residence**

13.00–14.00 **LUNCH AND TRADE EXHIBITION**

▶ PLENARY ◀ **14.00–15.00** **Dining Area · Castle Leazes Halls of Residence**

POSTER VIEWING AND TRADE EXHIBITION
CATEGORIES
Autopsy and Forensic **[39, 41–44]**
Breast **[97, 99–112]**
Head and Neck **[113–115]**
Hepatobiliary/Pancreas **[116–124]**
Neonatal/Paediatric **[125–143]**
Neuropathology/Ophthalmic **[144–150]**
Skin **[151–153]**

▶ PLENARY ◀ **15.00–16.00** **David Shaw Lecture Theatre**

PLENARY ORAL SESSION
Chair: Professor M Pignatelli, University of Bristol and Bristol Royal Infirmary
Dr BF Warren, John Radcliffe Hospital, Oxford

15.00 **[13]** ***Amplifications of the epidermal growth factor receptor gene (egfr) are associated with tumor progression in phyllodes tumours***
{P} H Buerger, C Kersting, J Packeisen, H Schmidt, PJ van Diest, A Kuijpers, W Boecker, B Brandt

15.15 **[14]** ***Application of the Royal College of Pathologist's (RCPath) workload guidelines to a specialist reporting system***
{P} M Ashton-Key

15.30 **[15]** ***Overexpression of the SEPT9_v4 ISOFORM confers resistance to microtubule-interacting drugs***
{P} SEH Russell, A Chacko, SS McDade, S Chanduloy, R Kennedy, S Church, PA Hall

15.45 **[16]** ***Achaete-Scute Like 2 (ascl2) is a target of Wnt Signalling and is upregulated in intestinal neoplasia***
{P} AM Jubb, S Chalasani, GD Frantz, V Kavi, HI Grabsch, NJ Maughan, P Quirke, H Koeppen

16.00–16.30 **Medical School Foyer**

16.00–16.30 **TEA**

▶ PLENARY ◀ **16.30–17.00** **David Shaw Lecture Theatre**

PLENARY ORAL SESSION
Chair: Professor M Pignatelli, University of Bristol and Bristol Royal Infirmary
Dr BF Warren, John Radcliffe Hospital, Oxford

16.30 **[17]** ***Molecular associations of cutaneous fatty acid binding protein in head and neck squamous carcinoma***
W Gomaa, Y Ke, H Fujii, **{P}** TR Helliwell

16.45 **[18]** ***Differentiation between clear cell sarcoma and malignant melanoma using a molecular approach***
{P} L Lam, T C Diss, F Berisha, B D Idowu, R E Gale, A M Flanagan

▶ PLENARY ◀ **17.15–18.00** **David Shaw Lecture Theatre**

THE BRITISH DIVISION OF THE INTERNATIONAL ACADEMY OF PATHOLOGY'S 1ST GEORGE CUNNINGHAM LECTURE
[241] ***The modern management of the patient with breast cancer. A celebration of the role of the pathologist***
Prof CW Elston, City Hospital, Nottingham
Chair: Prof NA Shepherd, Gloucestershire Royal Hospital

▶ PLENARY ◀ **18.45–20.30** **Alnwick Suite · St James's Park**

SYMPOSIUM AND RECEPTION: ***Recent Advances in GISTs*** – Sponsored by Novartis Pharmaceuticals
Chair: Dr BF Warren, John Radcliffe Hospital, Oxford

Numbers are limited – delegates who have reserved a place will receive a copy of the programme on arrival in Newcastle.

THURSDAY 7 JULY

▶ PARALLEL ◀ **09.00–12.00** **Lecture Theatre C**

SLIDE SEMINAR: ***Transplant Pathology***
Chair: Prof AD Burt, University of Newcastle-upon-Tyne
Dr DR Davies, John Radcliffe Hospital, Oxford

09.00–09.15 Dr F Black, Newcastle-upon-Tyne Hospitals NHS Trust, Newcastle

09.15–09.30 Dr D Rassl, Papworth Hospital, Cambridge

09.30–10.00 Dr M Burke, Harefield Hospital, Middlesex

10.00–10.30 Prof SG Hubscher, University of Birmingham

10.30–11.00 **Coffee** (Medical School Foyer)

11.00–11.15 Prof AD Burt, University of Newcastle-upon-Tyne

11.15–11.30 Dr K Wood, Newcastle-upon-Tyne Hospitals NHS Trust, Newcastle

11.30–11.45 Dr LJ McWilliam, Manchester Royal Infirmary

11.45–12.00 Dr MK Bennett, Newcastle-upon-Tyne Hospitals NHS Trust, Newcastle

▶ PARALLEL ◀ **09.00–10.30** **David Shaw Lecture Theatre**

ORAL COMMUNICATIONS: ***Breast and Cellular/Molecular Pathology***
Chair: Dr AJ Howat, Burnley General Hospital
Dr AJ Arends, Addenbrooke's Hospital, Cambridge

09.00 **[19]** ***Breast carcinomas with basal/myoepithelial differentiation: a review of morphology and immunophenotypical analysis***
{P} E Rakha, T Putti, D Abd El-Rehim, EC Paish, IO Ellis

09.15 **[20]** ***Influence of different mechanisms of chromosomal 16q-loss in invasive breast cancer on differential gene expression***
{P} H Buerger, E Korsching, D Kemming, AM Cleton-Jansen, W Boecker, B Brandt

09.30 **[21]** ***Better survival and distinguishing pathological features of breast cancer in patients with BRCA-1 germline mutations***
{P} B Sethi, P Makhija, KP Sidhu, K Ryder, H Hamed, S Hodgson, C D'Arrigo

09.45 **[22]** ***The prognostic and predictive significance of Oestrogen Receptor Beta in lobular carcinoma in situ***
{P} PS Young, SR Krivinskas, EC Paish, AR Green, IO Ellis

10.00 **[23]** ***Development of geminin mimetics as novel anti-proliferative agents***
{P} K Stoeber, KL Eward, SR Kingsbury, AL Okorokov, D Madge, DL Selwood, GH Williams

10.15 **[24]** ***SEPT6: genomics, transcripts, expression profile and evidence of deregulated expression in lymphoma***
{P} SEH Russell, CB Todd, M Dittani, H Grabsch, KJ Hillan, PA Hall

▶ PARALLEL ◀ **09.00–10.30** **Lecture Theatre D**

ORAL COMMUNICATIONS: ***Gastrointestinal and Hepatobiliary/Pancreas***
Chair: Prof GT Williams, University of Wales, Cardiff
Dr RFT McMahon, University of Manchester

09.00 **[29]** ***The expression of Interleukin-8 (IL-8) and IL-8 Receptors, CXCR1 and CXCR2 in colorectal carcinoma***
{P} Y Sadler, M Alison, MA El-Bahrawy

09.15 **[30]** ***Monoclonal conversion in human gastric glands gives insights into stem cell and clonal architecture***
{P} S McDonald, S Leedham, S Preston, D Oukriff, M Novelli, D Turnbull, J Jankowski, N Wright

09.30 **[31]** ***MCM Proteins and geminin in gastric cancer: association with prognosis, histological type, DNA Ploidy and proliferation***
{P} J Knock, M Shah, J Wyatt, M Dixon, G Casali, F Lewis, I Martin, A Jubb, K Stoeber, M Loddo, G Williams, P Quirke, H Grabsch

09.45 **[32]** ***Retrospective analysis of gastrointestinal soft tissue tumours in one UK regional referral centre: frequency and characteristics of gastrointestinal stromal tumours (GIST)***
{P} BA O'Sullivan, NS Deshmukh, GM Reynolds, I Geh, P Taniere

10.00 **[33]** ***The histology of autochthonous hepatitis E virus infection***
{P} P. Malcolm, H. Dalton, S.H. Hussaini, J. Mathew

10.15 **[34]** ***Prognostic significance of steatosis and intrahepatic invasion in patients undergoing liver resection for metastatic adenocarcinoma***
PJ Nisar, **{P}** AM Zaitoun, M Ethunandan, CC Dowson, SD Ryder, GP Aithal, J Doran, IJ Beckingham, DN Lobo

10.30–11.00 **Medical School Foyer**

COFFEE

▶ PARALLEL ◀ **11.00–12.00** **David Shaw Lecture Theatre**

ORAL COMMUNICATIONS: ***Education & Audit and Osteoarticular/Soft Tissue Pathology***
Chair: Prof WG McCluggage, Royal Group of Hospitals Trust, Belfast
Prof AM Flanagan, Royal Free and University College, London

11.00 **[25]** ***An e-learning system using virtual slides***
{P} M Waterhouse, H Dee, D Treanor, N Griffin, M Dixon, P Quirke

11.15 **[26]** ***A searchable online database of virtual slides***
{P} H Dee, D Treanor, F Lewis, G Casali, S Edward, M Dixon, P Quirke

11.30 **[27]** ***Histopathology E-recruitment in practice: experiences from year one national training schools recruitment 2005***
{P} P Naik, S Cossins, NR Griffin

11.45 **[28]** ***Detection of a new mutation in fibrous dysplasia using a modified site directed mutagenesis methodology***
{P} M Al-Adnani, B Idowu, L Yu, TC Diss, L Lam, R Gale, E O'Dell, A Flanagan

▶ PARALLEL ◀ **11.00–12.00** **Lecture Theatre D**

ORAL COMMUNICATIONS: ***Experimental Tumour Pathology, Technical Advances and Lymphoreticular Pathology***
Chair: Prof P Quirke, University of Leeds
Prof K Henry, Imperial College, School of Medicine, London

11.00 **[35]** ***The septin-binding protein anillin is over-expressed in diverse human tumours***
{P} PA Hall, CB Todd, PL Hyland, SS McDade, M Datani, H Grabsch, KJ Hillan, SEH Russell

11.15 **[36]** ***Using telepathology for rapid assessment of fine needle aspirates in the one-stop breast clinic***
U Chandran, **{P}** V Foria, N Singh, R Howitt

11.30 **[37]** ***Grade, BCL2 gene abnormality and expression, and clinical relevance in follicular lymphoma***
{P} JR Goodlad, PJ Batstone, D Hamilton, L Forsyth, MJ Sales, NR Pratt, NM Kernohan, DA Levison, JM White

11.45 **[38]** ***Follicular lymphomas lacking t(14;18)(q32;q21) constitute a clinicopathological subtype***
{P} JR Goodlad, PJ Batstone, D Hamilton, NM Kernohan, DA Levison, JM White

THURSDAY 7 JULY *continued*

▶ PLENARY ◀ **12.00–13.00** **David Shaw Lecture Theatre**

PATHOLOGICAL SOCIETY OF GREAT BRITAIN & IRELAND'S ANNUAL BUSINESS MEETING
(Members will have received an Agenda)

13.00–14.00 **Dining Area · Castle Leazes Halls of Residence**

LUNCH AND TRADE EXHIBITION

▶ PLENARY ◀ **14.00–15.00** **Dining Area · Castle Leazes Halls of Residence**

POSTER VIEWING AND TRADE EXHIBITION
CATEGORIES
Cellular/Molecular **[154–163]**
Education and Audit **[164–175]**
Endocrine **[176–178]**
Experimental Tumour Pathology **[179]**
Gastrointestinal **[180–207]**
Gynaecological **[87]** (Poster for ACEM Meeting – displayed in Lecture Theatre A)
Lymphoreticular **[208–215]**
Osteoarticular/Soft Tissue **[216–217 and 219–221]**
Technical Advances **[222–228]**

▶ PLENARY ◀ **15.00–17.00** **David Shaw Lecture Theatre**

SYMPOSIUM: ***Approaches to Macroscopic Pathology and Minimum Data Sets***
Chair: Prof GT Williams, University of Wales, Cardiff
Dr ME Mathers, Newcastle-upon-Tyne Hospitals NHS Trust, Newcastle

15.00–15.15 ***Introduction: Uses and abuses of minimum data sets***
Prof GT Williams, University of Wales, Cardiff

15.15–15.30 ***Approaches to complex urological cases***
Dr DR Davies, John Radcliffe Hospital, Oxford

15.30–15.45 ***Approaches to gynaecological specimens***
Dr S Manek, John Radcliffe Hospital, Oxford

15.45–16.00 **Tea** (Medical School Foyer)

16.00–16.20 **[242]** ***Approaches to neck dissections***
Dr TR Helliwell, Royal Liverpool University Hospital

16.20–16.40 **[251]** ***Approaches to BMS dissection and sampling of breast specimens – the Nottingham City Hospital experience***
Mr G Donovan, Nottingham City Hospital

16.40–17.00 ***Approaches to large bowel specimens***
Prof NA Shepherd, Gloucestershire Royal Hospital

▶ PLENARY ◀ **17.15–18.00** **David Shaw Lecture Theatre**

Pathological Society of Great Britain & Ireland's 3RD Doniach Lecture:
[243] ***Thyroid Carcinogenesis; Genotype-Phenotype correlation and the effects of radiation***
Prof Sir Dillwyn Williams, Strangeways Research Laboratory, Cambridge
Chair: Prof NA Wright, St Bartholomew's and The Royal London School of Medicine and Dentistry

19.30 (for 20.00) –23.00 **Alnwick Castle · Northumberland**

18.15 PROMPT! Buses leave from Medical School for Conference Dinner
19.30–23.00 **CONFERENCE DINNER**

THURSDAY 7 JULY *continued*

▶ PARALLEL ◀ 10.00–15.00 Fenwick Room & MGM Room · Castle Leazes Halls of Residence

COMPANION MEETING: UK NEQAS for Cellular Pathology Technique
Chair: Mr DW Evans, Newcastle-upon-Tyne Hospitals NHS Trust

10.00–10.20	**Chairman's Report** Mr D Evans, Newcastle
10.20–10.40	***Quality of assessment material and issues of consent*** Mr M Sutcliffe, Bournemouth
10.40–11.00	***Quality of assessors' performance*** Mr J Elsam, Lewes
11.00–11.30	**Coffee**
11.30–11.50	**Performance Review 2004–2005** Mr G Thompson, Nottingham
11.50–12.10	***Automated special stains*** Mr A Clark, Leeds
12.10–12.40	**Quiz** Mrs D Edwards, Sandwell
12.40–13.00	***EQA Schemes and Quality Management Systems*** Mr A Warford, Wellcome Trust Sanger Institute, Essex
13.00–14.00	**Lunch and Trade Exhibition** (Dining Area, Castle Leazes Halls of Residence)
13.00–14.00	**EQA Workshop** Mr P Jackson, Leeds
14.00–14.20	**Neuropathology review** Mr B McMeekin, Newcastle
14.20–14.35	**Quiz** Mrs D Edwards, Sandwell
14.35–15.00	***EQA and its importance to histological diagnosis*** – Sponsored by the Pathological Society of Great Britain & Ireland Prof AJ Freemont, University of Manchester
15.00	**Close**

THURSDAY 7 JULY *continued*

▶ PARALLEL ◀ **09.40–17.00** **Lecture Theatre A**

COMPANION MEETING: Association of Clinical Electron Microscopists

9.40–10.30 ***Unusual tumours needing electron microscopy for precise characterisation and diagnosis***
Dr B Eyden, Christie Hospital, Manchester

10.30–11.00 **Coffee** (Medical School Foyer)

11.00–11.50 **[249]** ***From the basement up – Ultrastructural clues in hereditary skin disorders epidermolysis bullosa and ichthyosis***
Ms T Dopping-Hepenstal, St John's at St Thomas' Hospital, London

12.00–12.30 **[250]** ***Ultrastructural telepathology – Possibilities and limitations***
Dr J Schroeder, University Hospital Regensburg, Germany

12.30–13.00 **Short presentation or poster viewing**

13.00–14.00 **Lunch** (Dining Area, Castle Leazes Halls of Residence)

14.00–14.50 ***EM and biological medicines (production and safety of viral vaccines)***
Dr R Fleck, National Institute of Biological Standards and Control, London

14.55–15.45 ***Electron microscopy in ophthalmic pathology***
Dr R Bonshek, Manchester Royal Infirmary

15.45–16.15 **Tea** (Medical School Foyer)

16.15–17.00 **Annual General Meeting** – *open to ACEM members and non-members*
Chair: Dr T Ryder, Charing Cross Hospital, London

FRIDAY 8 JULY

▶ PLENARY ◀ **09.00–12.00** **David Shaw Lecture Theatre**

SYMPOSIUM: ***Controversies in Cellular Pathology***
Chair: Prof GT Williams, University of Wales, Cardiff
Prof AD Burt, University of Newcastle-upon-Tyne

09.00–09.20 ***Specialisation in Cellular Pathology – for***
Dr BF Warren, John Radcliffe Hospital, Oxford

09.20–09.40 **[244]** ***Specialisation in Cellular Pathology – against***
Dr H Cochrane, Sunderland Royal Hospital

09.40–10.00 **Discussion**

10.00–10.20 ***MDTs are an effective means of handling patient care – for***
Prof RR Hall, Newcastle-upon-Tyne

10.20–10.40 **[245]** ***MDTs are an effective means of handling patient care – against***
Dr JT Roberts, Northern Centre for Cancer Treatment, Newcastle General Hospital

10.40–11.00 **Coffee** (Medical School Foyer)

11.00–11.20 **[246]** ***Autopsy practice should be an essential part of postgraduate training in pathology – for***
Dr PJ Gallagher, Southampton General Hospital

11.20–11.40 **[247]** ***Autopsy practice should be an essential part of postgraduate training in pathology – against***
Prof SB Lucas, St Thomas' Hospital, London

11.40–12.00 **Discussion and close**

12.00–13.00 **Medical School Foyer**

12.00–13.00 **TAKE-AWAY LUNCH**

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ABSTRACTS

1

Angiogenesis Markers In Ovarian Cancer Act As Prognostic Markers But Not Indicators Of Chemotherapy Response

{P} S O'Toole[1], BL Sheppard[1], JJ O'Leary[1], O Sheils[1], T D'arcy[2], N Gleeson[2], E McGuinness[2], A Laois[1], J Bonnar[1]
[1]. Trinity College Dublin, Dublin, Ireland, [2]. St. James's Hospital, Dublin, Ireland

The aim of this study was to assess two candidate markers, CD31 (a measure of microvessel density) and VEGF (vascular endothelial growth factor) as potential prognostic markers or indicators of response to chemotherapy in ovarian cancer.

Seventy nine ovarian specimens were analysed by immunohistochemistry. Pearson correlation, 1-way ANOVA and Chi Square were used for univariate analysis. Kaplan Meier survival curves were used, log rank and Cox proportional hazards regression model were used for analysis.

Increased staining with CD31 was associated with decreased progression free survival (PFS) ($p<0.01$) and overall survival (OS) ($p<0.01$) in univariate analysis. CD31 offered no indication as to which patients might best respond to chemotherapy. Quantifying VEGF proved to be a valuable independent prognostic indicator in PFS ($p<0.05$) and OS ($p<0.0001$) but fails to provide an adequate measure for resolving the response to chemotherapy.

CD31 and VEGF do play a role in some ovarian malignancies but other factors are likely to be involved. Only VEGF proved valuable as an independent prognostic marker in multivariate analysis. None of the markers were accurate in predicting chemotherapy response. Perhaps molecular profiling of tumours will determine which factors will be important for determining the response of the tumours to chemotherapy.

2

Histomorphometric Characterisation Of Shared And Non-Shared Cotyledonary Villus Territories In Monochorionic Placentae In Relation To Pregnancy Complications

{P} LY Wee[1], NJ Sebire[2], M Sullivan[1], NM Fisk[1]
[1]. Institute of Reproductive and Developmental Biology,Imperial college London, London, United Kingdom, [2]. Department of Histopathology, Great Ormond Street Hospital, London, United Kingdom

Abstract

Objective: To characterize the histomorphometry of terminal villi in shared and non-shared cotyledons in uncomplicated monochorionic placentae and those complicated by twin-twin transfusion syndrome (TTTS) or discordant growth restriction (ΔIUGR).

Methods: Histomorphometric variables of fifty terminal villi from each of twin's territory and shared cotyledons obtained by standard histological sectioning, were correlated with birthweight discordance, placental territory discordance and ΔAVAs [no. of arterio-venous anastomoses (AVA) from smaller (donor) to larger twin (recipient) - no of AVAs from larger to smaller twin].

Results: Histomorphometric variables were similar in shared and non-shared cotyledons of uncomplicated MCDA placentae. However median diameter of terminal villi in shared cotyledons in ΔIUGR and TTTS placentae were significantly smaller, had similar number of smaller capillaries, larger fetomaternal diffusion distance and reduced vascularization compared to non-shared IUGR and TTTS placentae. In ΔIUGR, $\log10\Delta\text{diameter}=1.38+(0.01*\text{birthweight discordance})+(0.56*\log_{10}\text{placental territory discordance})$ ($R^2=0.82$, $p<0.001$)(Δ diameter = the difference between median diameters of terminal villi in large - small twins' territories). In the TTTS group, Δdiameter correlated significantly with ΔAVA only: $\log_{10}\Delta\text{diameter} = 1.44+(0.02*\Delta\text{AVA})$ ($R^2=0.3$, $p<0.001$).

Conclusions: Abnormal placentation may be responsible for ΔIUGR in MC twins, whereas TTTS arises from imbalance in interfetal transfusion with resultant differing terminal villus histomorphometric features of donor, recipient and shared cotyledons.

3

An Immunohistochemical Comparison Between Low Grade and High Grade Ovarian Serous Carcinomas

{P} CJ O'Neill, MT Deavers, A Malpica, H Foster, WG McCluggage
[1]. department of pathology, institute of clinical sciences, royal victoria hospital, belfast, United Kingdom, [2]. dept. of pathology, belfast city hospital., belfast, United Kingdom, [3]. dept. of pathology, university of texas, MD Anderson Cancer Centre, Houston., houston, texas, United States

Supported by a grant from the Pathological Society of Great Britain and Ireland

Recently, a dualistic pathway of ovarian serous carcinogenesis has been proposed in which low grade ovarian serous carcinoma (OSC) arises in a stepwise fashion from a benign serous cystadenoma through a usual serous borderline tumour through a micropapillary variant of serous borderline tumour. In contrast, the more common high grade OSC arises de novo from the ovarian surface epithelium. In this study, we have investigated the immunohistochemical expression of a wide range of proteins in cases of low grade (n=22) and high grade (n=47) OSC. Antibodies used were p53, MIB1, BCL2, WT1, HER-2/neu, C-KIT, osteopontin and survivin. There was a statistically significant higher expression of p53, MIB1, BCL2, HER-2/neu and C-KIT in high grade compared to low grade OSC ($p < 0.05$). Osteopontin and survivin expression was variable with no significant difference in expression between low grade and high grade OSC. Although expression of both HER-2/neu and C-KIT was significantly higher in high grade compared to low grade OSC, only rare cases exhibited strong positivity with these antibodies, which could be of therapeutic value in individual cases. The significant differences in protein expression between low grade and high grade OSC provides further support for a different underlying pathogenesis. In particular, the differences in p53 immunoreactivity are in keeping with observations that p53 gene mutation is more common in high grade than in low grade OSC.

4

The Quality Of Autopsy Reports 1994-2004 – Do We Meet The RCPath Standards?

{P} R J Delaney, I S D Roberts
Department of Cellular Pathology, Oxford Radcliffe Hospitals NHS Trust, John Radcliffe Hospital, Oxford, United Kingdom

Good quality autopsy reports are important so that sufficient information is conveyed to Coroners, clinicians and relatives, especially with regard to causes of death and clinico-pathological correlations.

In order to determine the quality of autopsy reporting in our unit, we made a retrospective review of the first 100 Coronial autopsies in 1994, 1999 and 2004. The reports were compared against the standards outlined in the RCPath Guidelines on Autopsy Practise 2002.

Improvements have been made in the reporting of height and weight (62% 2004, 1% 1999, 0% 1994). The CNS was examined less frequently in 2004 (70% 2004, 100% 1999 & 1994). In those cases examined, the meninges were commented on more frequently (91% 2004, 74% 1999, 65% 1994). There were also improvements in the reporting of cerebral vessels, pulmonary vasculature, oesophagus and breasts. Major systemic veins and musculoskeletal system were less widely reported in 2004.

Less histology blocks were taken in 2004 (62% 2004, 97% 1999, 93% 1994). The proportion reported, however, is increasing, although further improvements should be made (42% 2004, 5% 1999, 9% 1994). The number of uncut histology blocks remains unacceptably high (48% 2004).

Clinico-pathological correlates are given more frequently but still less often than desirable (54% 2004, 38% 1999, 13% 1994). The rates of formulation errors in causes of death show no significant differences (9% 2004, 5% 1999, 6% 1994).

Our study shows some important improvements in the quality of autopsy reports following the introduction of guidelines, and highlights the need for further improvements in key areas.

5

Post Mortem Bacteriology: A Re-evaluation

{P} J A Morris, L M Harrison, S M Partridge
Department of Pathology, Lancaster, United Kingdom

Aim: To assess the value of post mortem bacteriology in autopsy practice; with specific emphasis on bacterial invasion of blood and cerebrospinal fluid (CSF).
Methods: A review of published articles. Studies were selected to cover the full age range including the perinatal period, infancy and adults. Priority was given to larger studies, those emphasising stringent asceptic techniques, those with a short and defined post mortem interval, and those presenting data on the relation of specific isolates to evidence of infection.
Data: The review covers over 5000 autopsies mainly in adults but including 1108 perinatal cases and 468 cases of sudden unexpected death in infancy (SUDI). Data are available on 4992 blood cultures, 743 cultures of spleen and 1168 CSF cultures.
Findings: Studies in which careful precautions have been used to reduce contamination show that approximately 2/3 of blood cultures are negative, 2/9 yield a single isolate and 1/9 have mixed growth. This applies in adult autopsies, in the perinatal period and in infants. The post mortem interval has only a small effect on the isolation rate. A pure growth of a known pathogen has a more than 50% likelihood of being found in association with genuine infection in adults and in the perinatal period. This review casts doubt on the importance of agonal spread; mixed cultures only occur in a minority of cases and are mainly due to contamination. Post mortem translocation is not a problem if the body is stored at 4 degrees centrigrade; this applies even if the post mortem interval is a few days. Cultures of CSF are usually sterile if obtained with careful technique. There is no convincing evidence that agonal spread from mucosal surfaces into the CSF can occur.
Conclusions: The main post mortem artefact is contamination not agonal spread or post mortem translocation. Contamination can be reduced by careful technique; therefore post mortem cultures can provide useful information. A pure growth of a pathogen is likely to be a true positive at all ages; but corroboration should be sought.

6

Should Facial Protection Be Worn During Post-Mortem Examination?

{P} M.R Rao, C Phelan
University Hospital of North Staffordshire, Stoke - on - Trent, United Kingdom

Aim: To assess the extent of macroscopic and microscopic blood splashes during evisceration and dissection at post mortem examination.
Methods: 80 surgical face masks from consultants, technicians and SHOs were examined for macroscopic and microscopic (dissecting microscope) blood splashes.
Results: Of the 80 masks examined, 65% had macroscopic blood splashes and 77.5% had microscopic blood splashes. The average number of all blood splashes occurring on face masks was higher during evisceration (3.9) than dissection (2.8). The average number of microscopic blood splashes occurring on trainees' masks was significantly higher compared to consultants' masks (2.7 v 1.9 p=0.04) and also significantly higher compared to technicians' masks (3.1 v 2.3 p=0.03). The macroscopic splashes also showed a similar trend (trainee v consultant: 1.8 v 0.9 p = 0.02 and trainee v technician: 2.6 v 1.6 p =0.01).
Conclusion: The number of blood splashes invisible to the naked eye, on the face masks is unexpectedly high and highlights a potential health risk to those not wearing facial protection. The trainees are more prone possibly due to their inexperience. We strongly recommend that all pathologists wear equipment protecting the face, preferably visors as recommended by the Health Services Advisory Committee.

7

Immunohistochemistry For C4d In Renal Allograft Biopsies: Which Method Should Be Used?

{P} ISD Roberts, LJ McWilliam, A McEwen, D Koo, I Quiroga, J Procter, MCNM Barnardo, M Sutton, L Cerundolo, DR Davies, PJ Friend, PJ Morris, SV Fuggle
[1]. Oxford Radcliffe Hospitals, Oxford, United Kingdom, [2]. Central Manchester and Manchester Children's University Hospitals, Manchester, United Kingdom

Staining for C4d is widely used as a marker of humoral rejection. Two methods are currently employed; monoclonal antibody (mAb) in frozen sections and polyclonal antibody (pAb) in paraffin sections. In this study we compare their specificity by using both methods in the same early protocol biopsies and correlating C4d-positivity with histology and circulating donor-reactive antibodies (DRA).

Two groups of patients were studied: group 1: 48 consecutive day 7 protocol biopsies (41 cadaveric donor, 7 living donor kidneys); group 2: 24 protocol biopsies, at implantation (pre- and post-perfusion) and day 7 post-transplantation, from 8 consecutive recipients of non-heart beating donor (NHBD) kidneys.

Group 1: 13/48 (27%) biopsies showed C4d positivity; 6 biopsies showed diffuse mAb positivity (mAb+), none focal mAb positivity, 6 diffuse pAb positivity (diff pAb+) and 4 focal pAb+. The % of patients with detectable DRA was 0%, 83% and 40% for those whose biopsies were focal C4d+, diffuse C4d+ (mAb and pAb) and C4d- respectively.

Group2: 7/8 patients had delayed graft function and all day 7 protocol biopsies showed severe acute tubular necrosis (ATN); none showed rejection. Implantation biopsies in one patient were mAb+; all were pAb-. All day 7 biopsies were mAb-, but 4 were pAb+ (2 focal, 2 diffuse). No DRA was detectable in any patient.

We conclude that diffuse C4d staining of peritubular capillaries with both polyclonal and monoclonal Abs have high specificity for humoral rejection, as indicated by the association with DRA. However, positivity in the absence of DRA may occasionally be seen with the polyclonal Ab in severe ATN. Focal pAb positivity shows poor correlation with DRA and histological rejection.

8

Bone Marrow-Derived Cells Contribute To A Small Extent To Regeneration Of Renal Parenchyma After Injury

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Kidney is able to regenerate itself after acute injury with ischaemia or nephrotoxins. Emerging evidence suggests that adult bone marrow (BM) is a source of precursor cells involved in the regeneration process. We investigated whether BM-derived cells contribute to tubular regeneration in murine models of acute tubular injury. Irradiated wild type female mice were transplanted with whole BM from male syngeneic mice. Renal injury was induced 4 weeks later by unilateral ischaemia reperfusion (I/R). Donor-derived cells were detected by *in situ* hybridisation for the Y chromosome and their phenotype identified by histochemistry for *Lotus Tetragonolobus* Agglutinin, which is a proximal tubular epithelial marker. Engraftment in injured kidneys (3.2%±0.4) was significantly greater (p=0.0007) than in contralateral control kidneys (0.7%±0.3). Confocal imaging also confirmed these results. To further validate our study, we used other natural markers of phenotypic differentiation. Male mutant beige mice having abnormal giant lysosomal granules in proximal tubular cells were used as BM donors. Renal injury was induced by injecting $HgCl_2$ intraperitoneally into recipient female mice. Similar to I/R, tubular cell engraftment was significantly increased (p=0.0286) in injured recipients (1.2%±0.08) compared with control non-injured mice (0.0%). Occasional beige granules were also detected in the kidneys of recipients, as visualised by PAS staining or electron- or fluorescence- microscopy. In conclusion, we report that renal tubules are derived to a small percentage from adult BM cells that may serve in the repair of the kidney and show that tissue damage enhances tubular cell repopulation.

9

Antibody Mediated Rejection – Diagnosis And Correlation With Renal Graft Outcome

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Introduction: Antibody mediated rejection (AMR) is associated with graft failure, is difficult to recognise histologically and treat with conventional immunosuppression. New diagnostic criteria are now defined for AMR based partly on the immunohistochemical detection of C4d in biopsies.
Aims: The aims of this study were to use a method for C4d staining in paraffin sections and determine the association between C4d staining, donor specific antibodies (DSA), histological features and graft outcome.
Methods: 97 patients were biopsied for graft dysfunction and classified using Banff 97 criteria. Features suggestive of AMR were noted. Paraffin sections were stained with polyclonal antibody C4dpAb (Biomedica) using an immunoperoxidase technique. DSA in concurrent serum samples was determined by ELISA. Relevant clinical data were reviewed.
Results: Of the 97 cases, 18 showed diffuse C4d positivity in peritubular capillaries, 22 showed focal positivity and 57 were negative for C4d. The only patients to develop DSA(9) were in the diffuse group. There was a significant association between diffuse C4d staining, the production of DSA ($p<0.0001$) and subsequent graft failure ($p<0.01$).
Conclusion: Although the concurrent detection of DSA and C4d positivity is uncommon, outcome in this group is poor. This group may benefit from therapies directed at the humoral response.
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10

Bone Marrow-Derived Cells And Mesenchymal-Epithelial Transition In Kidney Fibrosis

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Interstitial fibroblasts play a central role in the development of kidney fibrosis, particularly by producing extracellular matrix components. We sought evidence that bone marrow (BM)-derived interstitial cells are actively involved in collagen production.

Wild type female mice were transplanted with BM from male transgenics expressing luciferase and beta-galactosidase reporter molecules under the control of the COL1A2 gene. Fibrosis was induced by unilateral ureteric obstruction.

BM-derived myofibroblasts were identified by Y-ISH and immunohistochemistry for alpha-SMA. Fibrosis was evaluated by ISH for collagen I mRNA. Reporter molecule expression was assessed by chemiluminescent assays and ISH.

Collagen I mRNA was increased in obstructed kidneys. Wild type mice had no significant luciferase activity. The obstructed kidneys of transgenic mice showed increased luciferase activity. In obstructed kidneys of BM-transplanted mice, 15% of SMA-positive interstitial cells were BM-derived, but luciferase activity was not increased. Evaluation of beta-galactosidase activity proved unreliable.

Ureteric obstruction in transgenic mice gave widespread peri-tubular expression of luciferase mRNA, which paralleled collagen expression. Very rare tubular cells expressed luciferase mRNA in BM-recipients.

BM-derived cells make a trivial contribution to collagen I synthesis in renal fibrosis, but the presence of COL1A2-driven reporter mRNAs in damaged tubular cells supports the mesenchymal-epithelial transition hypothesis for tubular regeneration.

11

Comparative expression analysis of Pax3 and Pax7 during myogenesis in the mouse embryo

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Aims:
In the initial myogenesis of the trunk muscles the transcription factors Pax3 and Pax7 are known to play a role. Here we want to elucidate whether these genes are required for the myogenic cell specification in the head and for the further myogenic process *per se*.
Materials and Methods:
Pax3, *Pax7*, *MyoD* and *Myogenin* proteins were immunohistochemically detected at embryonal stages E11-15 on adjacent paraffin sections using the respective monoclonal antibodies.
Results:
We present a systematic *in situ* analysis of spatiotemporal characteristics of the *Pax3* and *Pax7* gene expression in comparison to that of *MyoD* and *Myogenin*.
Discussion:
The observed patterns of expression suggest that *Pax3* is not involved in myogenesis in the head, and its post-somitic expression is restricted to the time of myoblast migration, while the *Pax7* pro-myogenic function is shared among all striated muscles. *Pax7* is known to be a myogenic inducer in the trunk while our findings show that it is not in the head. *Pax3* and *Pax7* therefore cannot simply be considered to play similar roles in muscle development in the adult and embryo respectively.

12

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH). Clinicopathological correlation.

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Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is a rare cause of small airways disease and clinicopathological data are limited. We retrospectively reviewed six patients with DIPNECH in order to characterise this entity further.
Patients (male =2, female = 4) presented in two groups; those with slowly progressive dyspnoea referred to respiratory physicians, and those with lung nodule(s) on chest radiography referred for a surgical opinion. One patient had MEN1 syndrome. There was no association with tobacco smoking. Two patients showed a lymphocytosis on bronchoalveolar lavage (31% and 30%). Lung function tests were either normal or obstructive.
HRCT showed pulmonary nodules (multiple n=4, solitary n=1) with features of constrictive bronchiolitis (mosaicism (n = 3), airway dilatation (n = 2), and bronchial wall thickening (n=1)).
Histology showed focal obliterative bronchiolitis associated with neuroendocrine cell hyperplasia (NEH). Typical carcinoids were present in 3 cases. In one of these cases there was, in addition to typical carcinoids, an atypical carcinoid. This patient had MEN1 syndrome. Staining for CD45, CD3 and CD20 showed mild chronic bronchiolitis in all cases. TTF1 was weakly positive in foci of NEH (all 6 cases).
Follow-up from time of diagnosis ranges from 6 months to 6 years (mean 3 years). In those with respiratory symptoms, the interval between onset of symptoms and diagnosis ranged from 4 to 28 years (mean 15.5 years). Two patients died, one from a separate disease and one post-transplantation at 5 years. Three patients were clinically stable and one patient showed progression of disease.
DIPNECH is a slowly progressive disorder with variable presentation. The combination of nodules and mosaicism on HRCT with or without obstructive lung function should prompt consideration of this rare diagnosis. The presence of a lymphocytosis on BAL and associated mild bronchiolitis raises the possibility that anti-inflammatory therapy may be beneficial in progressive cases.

13

Amplifications of the Epidermal Growth Factor Receptor gene (*egfr*) are associated with tumour progression in phyllodes tumours

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Phyllodes tumours of the breast are rare biphasic tumours with the potential for invasion and metastatic spread. An important role of the Epidermal Growth Factor Receptor (EGFR) in phyllodes tumours has been proposed. However, detailed pathogenetic mechanisms remained unclear.

We investigated 58 phyllodes tumours of the breast (40 benign, 10 borderline and 8 malignant) by means of *egfr* fluorescence in-situ hybridization (FISH) and gene dosage PCR for a regulatory sequence within intron 1 of *egfr*. Immunohistochemical staining was performed for EGFR, p16, p21, p27, p53, c-myc, Cyclin A, Cyclin D1, Cyclin E, c-kit and Ki-67.

Immunopositivity for EGFR in stromal tumour cells, but not in the epithelial component was detected in 19% (75% of all malignant tumours). Whole gene amplifications were seen by FISH in 15.8% (in stromal cells only) and intron 1 amplifications by gene dosage PCR in as much as 41.8% of all phyllodes tumours. Significant correlation were seen between tumour grade and EGFR overexpression ($p=0.001$) and intron 1 amplifications ($p<0.05$). EGFR overexpression further correlated with positive immunohistochemical staining for p53, p16, Cyclin A, Cyclin E, Ki-67 and c-kit. Presence of intron 1 amplification correlated with p16 ($p<0.01$), p21 ($p=0.009$) and p53 immunoreactivity ($p<0.001$). No EGFR immunopositivity nor whole gene amplification was observed in a complementary series of 167 fibroadenomas and only one of 43 (2.3%) exhibited intron 1 amplification in gene dosage PCR.

In conclusion, our results show for the first time that activating mutations in and overexpression of *egfr* are associated with the progression of phyllodes tumours of the breast.

14

Application Of The Royal College Of Pathologist's (RCPath) Workload Guidelines To A Specialist Reporting System

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In 2003 the RCPath published new guidelines on staffing and workload for cellular pathology departments[1]. This study assessed the application of these guidelines. In January 2004 average hours of work for each speciality team was agreed based on team consensus. Since January 2004 all specimens received by the Cellular pathology department of the Author were allocated a speciality code. Following reporting, specimens were given both macroscopic and microscopic workload codes using the RCPath guidelines. Computer searches for a year were undertaken for all speciality and workload codes. From these data, workload in terms of hours per week was calculated. Results: 26370 specimens were received and 24855 (94%) had been appropriately coded. For paediatrics, ophthalmic, lung and liver the estimated hours using the RCPath codes was equivalent to the departmental consensus. The RCPath codes overestimated the time required for skin, urology, bonemarrow and gastrointestinal specimens and underestimated the time required for gynaecological, breast, lymphoma and ENT specimens compared to our in-house consensus. Conclusions: The RCPath workload guidelines need revision to accurately reflect all specialities. Workload can be readily assessed using simple SNOMED type codes.

1)http://www.rcpath.org/resources/pdf/Sept04_StaffingWorkload_histo_cyto_depts.pdf

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OVEREXPRESSION OF THE SEPT9_v4 ISOFORM CONFERS RESISTANCE TO MICROTUBULE-INTERACTING DRUGS

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SEPT9 is one of at least 13 septin family members. This family of genes is highly conserved and encodes proteins involved in a variety of cellular functions including cytokinesis, apoptosis and vesicle trafficking. Septin co-localisation with actin and tubulin has also been reported. The *SEPT9* gene on chromosome 17q25.3 has a complex genomic architecture such that up to 18 different transcripts are possible by shuffling of six 5' ends and three 3' ends. Two of the 5' ends (*SEPT9_v4* and *SEPT9_v4**) are distinct transcripts with different 5'UTR sequences but encode the same protein. We have shown that elevated levels of *SEPT9_v4** mRNA are common in sporadic ovarian tumours with reduced expression of *SEPT9_v4* transcripts and that the translational efficiency of *SEPT9_v4** is enhanced relative to that of *SEPT9_v4*. Taken together these data argue that deregulation of *SEPT9_v4* occurs in neoplasia with enhanced translation of SEPT9_v4 protein. We are therefore concerned to determine the functional consequences of altered SEPT9_v4 expression in epithelial cells and how this might contribute to aspects of the neoplastic phenotype. In this study, we show that over-expression of SEPT9_v4 confers resistance to microtubule but not DNA-interacting drugs. SEPT9_v4 over-expressing cells are more able to maintain the integrity of the microtubule network in response to treatment with paclitaxel or depolymerisation on ice. In addition, when allowed to repolymerise, the SEPT9_v4 over-expressing cells lines do so more slowly than control cells. Given that SEPT9 over-expression is seen in diverse tumours and in particular ovarian and breast cancer, such data indicate that SEPT9_v4 expression be clinically relevant and contribute to some forms of drug resistance.

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Achaete-Scute Like 2 (*ascl2*) Is A Target Of Wnt Signalling And Is Upregulated In Intestinal Neoplasia

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ASCL2 is a basic helix-loop-helix transcription factor, which is essential for the maintenance of proliferating trophoblasts during placental development. Using Affymetrix oligonucleotide microarrays (MAs) we identified *ascl2* as a gene significantly upregulated in large intestinal adenocarcinomas (n=36 cancers, n=16 normals; 15-fold, $P<0.0001$).

This finding was confirmed by quantitative (Q)RT-PCR on large (n=29 cancers, n=16 normals; 10-fold, $P<0.0001$) and small intestinal adenocarcinomas (n=6 normals and cancers; 5-fold, $P=0.06$). *In situ* hybridization (ISH) for *ascl2* demonstrated expression at the base of normal crypts (n=187), but in no other normal tissues excepting placenta. By ISH, 52-71% of colorectal adenomas (n=304), 50-73% of large (n=327) and 33-64% of small intestinal adenocarcinomas (n=124) were positive for *ascl2* expression. Upregulation of murine *ascl2* was also observed using MAs, QRT-PCR and ISH on $apc^{min/+}$ (n=21 tumours, n=15 normal) and $apc^{1638N/+}$ (n=6 tumours, n=4 normal) tumours.

Cell lines stably-transfected with $LEF1^{DN}$ or APC2, or transiently transfected with short-interfering RNA against β-catenin showed a significant downregulation of *ascl2* by QRT-PCR. An association between *ascl2* ISH and nuclear β-catenin (immunohistochemistry) was observed in 73 small intestinal adenocarcinomas ($P=0.0008$) and co-localization was observed in $apc^{min/+}$ tumours.

In summary, *ascl2* is over-expressed in intestinal neoplasia and is a target of Wnt signalling.

17

Molecular Associations of Cutaneous Fatty Acid Binding Protein in Head and Neck Squamous Carcinoma

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Cutaneous fatty acid binding protein (C-FABP) is widely expressed in head and neck carcinomas. C-FABP has also been associated with angiogenesis and metastasis in prostatic adenocarcinomas. This study investigates the expression of C-FABP in relation to markers of proliferation and angiogenesis in head and neck carcinomas.
Tissue microarrays (TMAS) were constructed from 100 primary squamous carcinomas and their nodal metastases. Immunocytochemical labelling was performed for C-FABP, Ki-67, PPAR-γ, and angiogenesis-related markers (VEGF, Flk-l, CD31, and COX-2). For a subset of 30 representative carcinomas, the labelling in the TMA was compared with that in whole tissue sections.
There was moderate or substantial agreement between TMA blocks and whole tissue sections for C-FABP, Ki-67 and VEGF (weighted kappa values 0.59, 0.48 and 0.67 respectively). C-FABP expression is not associated with the Ki-67 index or microvessel density. Significant positive correlations were seen between cytoplasmic C-FABP expression and VEGF, PPAR-γ (p <0.01) and Flk-l (p <0.05). Increased COX2 expression occurs in dysplasia and poorly-differentiated carcinomas.
TMA is a reliable technique for investigating these molecules in squamous carcinomas. Although C-FABP expression is not directly related to proliferation or vascular density, potentially important effects may be mediated by C-FABP through the PPAR-γ and VEGF/Flk-l pathways.

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Differentiation Between Clear Cell Sarcoma And Malignant Melanoma Using A Molecular Approach

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Metastatic malignant melanoma (MM) and clear cell sarcoma (CCS) are difficult to distinguish. Both are characterised by a packeted growth pattern of S100 and HMB45 positive epithelioid cells with large nuclei and prominent nucleoli. However, CCS are characterised by t(12;22)(q13;q12) involving EWS/ATF1 fusion whereas most MM harbour a BRAF *(*usually V599E) or KRAS mutation.

To determine the utility of detection of t(12;22) and BRAF mutation to distinguish CCS and MM, 23 cases of clear cell sarcoma from the RNOH archive were reviewed. DNA and RNA extracts from each case were analysed for the presence of EWS/ATF1 using RT-PCR and for BRAF V599E mutation using mismatch primer/restriction digestion, WAVE and sequence analysis.

EWS/ATF1 transcripts were identified in 10 cases using RT-PCR. Four cases without detectable EWS/ATF1 contained a BRAF mutation. None had both a BRAF mutation and EWS/ATF1 and nine exhibited neither EWS/ATF1 nor BRAF mutation. Those with BRAF mutation had clinical evidence of MM, such as widespread metastatic disease.

Thus a molecular approach can help distinguish between CCS and MM. Adoption of additional techniques such as FISH for detection of EWS rearrangements and demonstration of KRAS mutations may increase the detection rate and improve the distinction of these two entities.

19

Breast Carcinomas With Basal/Myoepithelial Differentiation: A Review Of Morphology And Immunophenotypical Analysis

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The aim of this study was to assess the morphological characteristics and immunohistochemical profile of breast carcinomas with basal/ myoepithelial phenotype. We have examined 1870 cases of invasive breast carcinoma, using tissue microarray technology and immunohistochemistry, to identify tumours that show basal/myoepithelial phenotype. Their immunophenotype profile was characterized using a variety of markers. In addition, H&E stained sections were studied for several morphological parameters. Tumours were classified into 2 groups; 1) tumours with basal phenotype [expressing ck5/6 and/or ck14]; 2) tumours with myoepithelial phenotype (expressing actin and/or p63). Group1 was further divided into two subgroups; A) dominant basal pattern [10-50% of cells are positive]; B) basal characteristics [≥50% of cells are positive]. Group1A tumours constituted 10%; group1B 8.6% and group2 constituted 13.7% of the cases. The majority of these tumours were grade 3. There were positive associations with adenoid cystic growth pattern, loss of tubular formation, marked cellular pleomorphism, poorer NPI, development of distant metastasis. Associations were found with loss of expression of hormone receptors, neuroendocrine markers, BRCA1 and Fhit proteins and positive expression of p53, EGFR and p-cadherin. No association with vascular invasion was found. The commonest histological types were ductal/no special type, medullary like and mucinous carcinomas. The most common morphological characters of group1 were bigger size, high-grade comedo-type necrosis, positive lymph node disease and development of tumour recurrence. Group2 was noticed in younger age group and associated with central necrosis/fibrosis, basaloid cell change, positive e-cadherin, cerbB-4. Group1 showed a strong negative association with both overall survival (OS) (Log Rank(LR)=22.5, p<0.001) and disease free interval (DFI) (LR=30.1, p<0.001) while group 2 showed an association with OS (LR=5, p=0.02) but not with DFI. Multivariate analysis showed that basal but not myoepithelial phenotype has an independent value in predicting outcome.

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Influence of different mechanisms of chromosomal 16q-loss in invasive breast cancer on differential gene expression

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Aims: Comparative genomic hybridization (CGH) demonstrated that well-differentiated breast tumors showed significantly more physical loss of chromosome 16q than did poorly differentiated ones. However, polymorphic markers detected no difference in the frequency of 16q-LOH between invasive tumors of different histological grade.
Methods: We combined data on LOH, fluorescence in situ hybridization with chromosome 16–specific probes, CGH and gene expression analysis (n=20) with and witout chromosomal 16q-losses using the ABI gene expression platform.
Results: We are able to show a preference in well-differentiated grade I tumors for physical loss of chromosome arm 16q, whereas in poorly differentiated grade III tumors LOH is accompanied by mitotic recombination. Differentially regulated genes in tumours with physical loss of 16q were predominantly located on chromosomes 16q. All differentially regulated genes have not been described before in the pathogenesis of breast cancer.
Conclusions: This clarifies the discrepancies observed between CGH and LOH for 16q in breast cancer. These different somatic genetic mechanisms may reflect the presence of multiple tumor suppressor genes or oncogenes with that are the target of LOH/physical loss at chromosome arm 16q. It becomes also obvious that the different, previously described pathogenetic pathways in breast cancer are associated with a specific differential expression of chromosomes 16q genes. Validation studies are currently underway.

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Better Survival And Distinguishing Pathological Features Of Breast Cancer In Patients With BRCA-1 Germline Mutations

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Patients with BRCA-1 germline mutation develop breast cancer at a young age and follow-up data suggest similar or worse prognosis than grade matched controls. BRCA-1 tumours often have distinguishing histopathology and this may be helpful for pre-selection to increase detection rates of genetic testing. To assess the validity of any distinguishing feature of BRCA-1 breast cancer and gain better understanding of its prognosis, we studied a cohort of 24 breast cancer patients with BRCA-1 mutation and long-term follow-up (upto 25yrs) and compared them to tumours matched for age at presentation, year of presentation and tumour grade.
Very high mitotic rates (>40/10HPF), absence or small amount of DCIS, negativity for ER and PR, pushing margins and peripheral distribution of lymphoplasmacytic infiltrate distinguished BRCA-1 tumours from controls. Metachronous contra-lateral primary breast cancer was common in BRCA-1 (50% v 9% in controls) and new primaries arose throughout follow-up. Despite this, overall survival was significantly better in BRCA-1 cases than in age and grade matched controls (92% v 57% at 5y; 83% v 53% at 10y; 66% v 45% at 15y; 53% v 35% at 20y; p= 0.044) or in a control group of 1911 grade III tumours treated at the Breast Unit from 1970-1999 (p= 0.0025).

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The Prognostic and Predictive Significance of Oestrogen Receptor Beta in Lobular Carcinoma *In Situ*

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Lobular carcinoma *in situ* is a putative, non-invasive precursor lesion of breast cancer. Oestrogen has a significant role in promoting the development and progression of breast cancer which is mediated through Oestrogen Receptors (ER). Studies of ER expression in LCIS are limited and the characterisation of ER-α and ER-β status in LCIS may potentially be used as a marker of predisposition to develop invasive breast carcinoma.
Six normal breast lobule samples and 55 LCIS samples were immunohistochemically stained and evaluated for protein expression of ER-α, ER-β and E-cadherin (CDH1). Six normal lobule samples and 11 LCIS samples were isolated using laser microdissection, total RNA was extracted, synthesised to cDNA and gene expression quantified by real-time PCR.
ER-α and ER-βcx expression were significantly higher in LCIS than normal lobules. There was no difference in ER-βwt gene expression or ER-β protein expression between LCIS and normal lobules. E-cadherin expression was significantly reduced in LCIS compared with normal lobules.
Up-regulation of ER-βcx and down-regulation of CDH1 in LCIS could indicate regulation by ER-α. ER-α and ER-βcx status in LCIS may therefore have potential for further study to determine whether their expression is related to predisposition of women diagnosed with LCIS to develop invasive breast carcinoma.

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Development of geminin mimetics as novel anti-proliferative agents

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The origin licensing repressor geminin provides an intriguing molecular link between the cellular circuits controlling proliferation, differentiation and genomic stability. To understand how this unique bi-functional molecule might co-ordinate proliferation and differentiation in human cells, we resolved geminin's structure by transmission EM of negatively stained protein samples and single-particle analysis. Our data provide the first molecular structure of full-length geminin, and show that the molecule is a tetramer formed by two dimers with monomers interacting via coiled-coil domains. Biochemical analysis of full-length and truncated geminin forms in a human *in vitro* replication assay in conjunction with peptide inhibition studies resulted in the identification of a 15 kDa fragment that is fully functional in inhibiting DNA replication initiation. The 15 kDa fragment lacking the destruction box sequence of the endogenous geminin gene was covalently coupled to a novel small molecule carrier which can transport polypeptides across cell membranes. Addition of SMC-Δgeminin to asynchronous cultures of normal and transformed human cells diminished chromatin loading of the replicative Mcm2-7 helicase, inhibited DNA replication, and resulted in the accumulation of cells in G1 phase. Since geminin mediated repression of origin licensing may result in cancer-specific cell killing, linkage of key functional domains to geminin's molecular blue print could form the basis of an alternative drug discovery programme for anti-proliferative small molecule regulators of DNA replication.

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SEPT6: genomics, transcripts, expression profile and evidence of deregulated expression in lymphoma

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An increasing body of data indicate that members of the septin gene family are implicated in human neoplasia. For example *SEPT9* lies at a locus that shows allelic imbalance in ovarian and breast cancer, is a target for retroviral insertional mutagenesis and is over-expressed in diverse human tumours and is a translocation partner for MLL in human leukaemia. SEPT6 has also been implicated in leukaemia as a fusion partner of MLL. We therefore investigated the genomic architecture, transcript profile, mRNA and protein expression of SEPT6. The 12 exons of *SEPT6* span 76.6 kbase on Xq24 and undergo complex splicing events. We defined these splicing events and report 7 distinct transcripts that encode 5 distinct polypeptides. Of note are 3 discrete transcripts encoding the same 427 amino acid polypeptide: a phenomenon also seen with *SEPT9*. We used a quantitative RT-PCR strategy to define the expression of these transcripts in cell lines and in fetal and adult tissues and show tissue specific patterns of expression. We took advantage of Affymetrix probe sets from sequences in exon 11a (212415_at), 11b (212413 _at, 212414 _at and 214298_x_at) and exon 12 (213666_at) and define *SEPT6* mRNA expression in normal, diseased and neoplastic human tissues (n=10360). The RT-PCR data validated the expression array and *SEPT6* is expressed predominantly in lymphoid and CNS tissues. These data are further substantiated by the use of anti-SEPT6 sera we generated that show that SEPT6 expression is high in these cell types. SEPT6 protein is associated with the microtubule network and we have also identified a novel nuclear form of SEPT6 and show this to be mediated by a bipartite NLS in the C terminus of SEPT6. Finally we show that SEPT6 is consistently over-expressed in lymphoid neoplasms (n=106).

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An e-learning system using virtual slides

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An interactive e-learning system has been created which uses virtual slides to support postgraduate histopathology training. The system allows trainees to actively view teaching cases as virtual slides on the internet and provides feedback to trainees.

The system is intended to supplement traditional multi-header microscope teaching sessions and to allow the development of lessons based on common diagnostic problems. Lessons can be graded according to difficulty to encourage the development of diagnostic skills.

A "virtual case conference" is created by the trainer using a web based interface consisting of a virtual slide together with a list of differential diagnoses. Virtual slides can be made from any conventional histopathological or cytological slides, allowing the use of existing teaching archives. Trainers will be able to annotate slides to guide a trainee to an area of interest or important diagnostic feature, and provide macroscopic images of specimens.

Trainees access the system using the internet. They view the virtual slides and choose from the list of differential diagnoses provided. The system provides feedback to the trainee, with arguments for and against a particular diagnosis. Interactive prompts are provided to guide the trainee to the correct diagnosis.

These "virtual case conferences" will be made into a library of tutorials available on the web which will cover important diagnostic problems in histopathology.

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A searchable online database of virtual slides

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Improvements in computing have made complete digitisation of histopathological slides possible. It is now possible to scan entire slides and view them remotely over the internet. The virtual microscopy project at the University of Leeds has created a repository of these "virtual slides".

These slides are essentially massive digital images, which can be navigated and viewed at any magnification (up to 40X objective) like a conventional microscope. In addition virtual slides can be annotated and labelled by the pathologist, and used in live telepathology consultations.

In the first six months of the project, more than 6,000 virtual slides have been created, requiring 1,400 gigabytes of storage space, and the virtual slide repository is continually growing as more slides are scanned. A method to organise these virtual slides is needed.

A web based database has been created which is capable of storing considerable detailed "metadata" about these slides. Slides can be searched by SNOMED code, organ system, diagnosis or many other criteria. For example, all slides from the colon, or all slides with a particular diagnosis, or all gastric biopsies.

This virtual slide repository differs from existing image databases in that for the first time in that it is large, searchable and features actual *virtual slides* rather than just snapshots of individual fields. Our database is presented as a model for virtual slide databases and its use is demonstrated.

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Histopathology E-Recruitment In Practice: Experiences From Year One National Training Schools Recruitment 2005

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INTRODUCTION: An e-recruitment solution for National Histopathology Training Schools (NHTS) was demonstrated at the 187th meeting of the Society.
METHODS: The NHTS website launched in September 2004 was the main tool for attracting applicants to Year One training posts, replacing traditional literature. A questionnaire was sent to all applicants between the closing date and short-listing to canvass their views of the site and assess their online behaviour.
RESULTS: There were 470 applicants to NHTS, an increase of 104% over the 2004 recruitment round. 248 (52.8%) responded to the questionnaire, of these, 84.6% resided in the UK. 43.5% accessed the web from home and work on average 5-10 hours per week and 94% downloaded their applications electronically. The number of short-listed applicants was increased by 83.6%, and of those appointed, 45% were UK graduates. An analysis of applicant demographics, electronic information preferences, web usage and other aspects of feedback will be presented.
CONCLUSIONS: An e-recruitment solution in Histopathology has been successfully implemented and well received by applicants. Feedback received in this exercise will enhance and optimise the web portal for 2006 recruitment.

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Detection of a new mutation in fibrous dysplasia using a modified site directed mutagenesis methodology.

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Fibrous dysplasia is a benign intramedullary fibro-osseous lesion, resulting from activating missense mutations in the GNAS! Gene. Known GNAS1 mutations occur in codons 201 (C→T or G→A) and 227 (A→T). We have compared the number of mutations detectable using a site- directed mutagenesis approach and direct DNA sequencing in gnathic and extra-gnathic sites.
46 extra-gnathic and 16 gnathic FD cases were studied. DNA was extracted from micro-dissected paraffin-embedded sections from decalcified specimens. Using a site-directed mutagenesis approach, 3 simultaneous PCR reactions for each case were performed to detect the 3 known mutations. The use of mismatch primers followed by digestion with the appropriate enzyme (Nco1, Dde1 or Stu1) discriminates between the wild-type allele (undigested) and mutant allele (undigested). DNA from the extra-gnathic FD cases was also analysed by direct sequencing.
PCR products were generated from all the extra-gnathic and from 13 gnathic FD cases for the site-directed mutagenesis method and from 39 extra-gnathic FD cases for sequencing. 31 mutations (67%), including 1 A→T mutation, were detected in the extra-gnathic FD cases, while 23 mutations (59%) were detected using direct DNA sequencing. Sequencing failed to detect mutations in 6 cases. Therefore, the new method showed a 15% improvement in mutation detection. Mutations were detected in 5 (38.5%) gnathic FD cases, including 1 A→T mutation.
In the largest study of its kind, we have developed a new site-directed mutagenesis method for the molecular diagnosis of fibrous dyspalsia that shows a 15% improvement in mutation detection over direct DNA sequencing. We have detected the first two codon 227 A→T mutations in fibrous dysplasia.

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The Expression of Interleukin-8 (IL-8) and IL-8 Receptors, CXCR1 and CXCR2 in Colorectal Carcinoma

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IL-8, a potent chemoattractant for neutrophils, is also implicated in mediating tumour growth and progression. We and others have demonstrated the expression of IL-8 and IL-8 receptors in colorectal carcinoma cell lines. The aim of this study was to investigate the expression of IL-8 and its receptors, in colorectal adenocarcinomas.
Fifty colorectal adenocarcinomas of different grades and stages were studied by the avidin-biotin complex immunocytochemical technique.
IL-8 immunoreactivity was detected in epithelial cells of only 3/50 tumours, but was strongly expressed in macrophages and some neutrophils in the stroma of all tumours. All tumours expressed CXCR1 at varying intensities in the membrane and cytoplasm of tumour cells. CXCR1 expression showed correlation with Dukes' stage, being strongest in Dukes' C tumours. CXCR2 was only weakly and focally expressed in occasional tumours.

This is the first in vivo study of IL-8 and its receptors in colorectal cancer. CXCR1 is overexpressed in colorectal carcinoma as compared to normal colonic mucosa, and appears to be the receptor through which the effects of IL-8 are mediated on colonocytes. CXCR1 may represent a potential immunotherapeutic target in advanced colorectal cancer.

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Monoclonal Conversion in Human Gastric Glands Gives Insights into Stem Cell and Clonal Architecture

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Introduction: Stem cell organisation of the human gastric gland has been an enigma. Studies in animals have shown that glands are clonally-derived: however, others have reported that a minority of glands remain polyclonal, suggesting multiple, possibly independent stem cells. Exploiting the relatively high mutation rate of mitochondrial (mt) DNA as compared to nuclear DNA, we used mt cytochrome c oxidase subunit 1 (COXsu1) as a clonal marker to determine gastric gland lineage relationships. **Hypothesis:** Human gastric glands are clonal structures which originate from multiple stem cells and expland by gland fission. **Methods:** Double enzyme histochemistry was performed on frozen serial sections of human gastric biopsies for reactivity of COXsu1 in conjunction with the nuclear-encoded respiratory chain enzyme succinate dehydrogenase (SDH), the principle being that any mutation in the mtDNA that leads to inactivity of the COXsu1 enzyme within a cell or gland will result in only SDH activity being detected. **Results:**We observed body-type gastric glands that are partially filled with mutated (COXsu1-, SDH+) cells, and other glands where *all the cells are mutated*. These data suggest that a stem cell bearing this mutation is able to expand, probably stochastically, to fill the entire gastric gland. Moreover, we have observed groups of wholly-negative glands, suggesting that these mutations can spread by gland fission. **Conclusions:**Gastric glands, like the intestinal crypts, undergo a process of monoclonal conversion, where the gland is replaced by mutated cells. The human gastric gland is thus maintained by a number of stem cells. Each gland is derived from a single stem cell that gives rise to all the contained lineages:gastric stem cells are therefore multipotential. Thus mutated glands expand by gland fission.

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MCM Proteins And Geminin In Gastric Cancer: Association With Prognosis, Histological Type, DNA Ploidy And Proliferation

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Minichromosome maintenance proteins (MCMs) and geminin play a vital role in initiation of DNA synthesis. Our RNA expression array data showed that MCMs but not geminin are upregulated in gastric adenocarcinomas (GC) and that these genes were differently expressed depending on histological subtype or stage. The pattern of protein expression of these important cell cycle regulators has not been characterised in GC.
We studied the expression of MCM2, MCM3, MCM5, MCM7 and geminin by immunohistochemistry on tissue microarrays constructed from 163 GC. The percentage of positively stained tumour cells was scored and its relationship with clinicopathological data, patient survival, Ki67 and DNA ploidy was analyzed. P values <0.05 were regarded as significant.
The expression of all proteins were positively correlated with Ki67 expression. Expression of MCM3, MCM5, and MCM7 was higher in DNA diploid GC supporting their role in maintaining euploidy. The unexpected survival benefit of high MCM5 and MCM7 expression may be explained by their additional role in the S phase DNA damage checkpoint and/or their interaction with transcription factors MYCN and STAT. Geminin's role as tumour suppressor seems to be supported by its relationship to patient survival and pT in GC. Higher expression of geminin in intestinal-type GC may indicate different regulation of DNA replication initiation in different histological subtypes. Further studies are now necessary to investigate the regulation of MCMs and geminin expression and to establish whether these markers may not only predict patients prognosis as shown in our study, but may also be of value in stratifying patients for more specific treatment.

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Retrospective Analysis of Gastrointestinal Soft Tissue Tumours in one UK Regional Referral Centre: Frequency and Characteristics of Gastrointestinal Stromal Tumours (GIST)

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Introduction
The aim of the study was to analyse all intra abdominal mesenchymal tumours removed at our institution in the last 15 years and to determine the frequency of GIST. 92 tumours were retrieved. All sections had their morphology reviewed by 2 pathologists. Immunostaining for SMA, Desmin, S100 protein, CD34, CD117 (KIT) and PDGFRA (Platelet-derived Growth Factor Receptor alpha) proteins was performed in all cases.
Results
54/92 (59%) cases were reclassified as GISTs. Eleven were leiomyomas, 19 were leiomyosarcomas, 3 were unclassified spindle cell tumours, 1 was fibromatosis, 1 was a solitary fibrous tumour, 1 was an angiomyolipoma and 2 were angiosarcomas. For GIST, M/F sex ratio was 1.16. Mean age at diagnosis was 61 years. The most common site was stomach (25 cases). 52/54 GISTs showed KIT expression. All 92 tumours were positive with PDGFRA marker.
Discussion
This is the first report on frequency of GIST among GI soft tissue tumours in the UK. At approximately 60% of cases, it is comparable to rates observed in other countries. Our study demonstrates that PDGFRA and KIT expression is not mutually exclusive in GIST and that PDGFRA protein is expressed in all abdominal soft tissue tumours.

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The Histology Of Autochthonous Hepatitis E Virus Infection

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Aim: To describe the histological appearance of autochthonous Hepatitis E Virus (HEV) infection.
Case Report: We describe four patients who presented with malaise, jaundice and epigastric discomfort. They all ate meat products and had positive HEV serology.
Histology showed mild distortion of the reticulin framework with `lobular' disarray, varying degrees of cholestatic rosette formation and, zone 3 cytoplasmic and canalicular bilirubinostasis. A striking acinar inflammatory infiltrate was present with spotty hepatocyte necrosis, apoptotic hepatocytes and focal microconfluent necrosis; this infiltrate was composed of lymphocytes, including plasma cells, but also a prominent polymorph (eosinophilic and neutrophilic) infiltrate. Marked hepatocyte anisonucleosis was noted. Three cases showed mild central venous fibrosis.
There was a marked portal lymphocytic inflammatory infiltrate with significant interface hepatitis and a cholangiolitis. The infiltrate was composed of lymphocytes, including plasma cells, forming occasional aggregates and polymorphs (eosinophils and neutrophils), similar to that in the acinus. Significant steatosis, Mallory's hyaline and megamitochondria were not present. There was no evidence of accumulation of iron, copper or alpha-1-antitrypsin.
Conclusion: The histological appearances of autochthonous HEV are striking and, in our opinion, diagnostic. We believe that it can be readily differentiated from other viral hepatitides and, cholestatic or autoimmune hepatitis.

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Prognostic significance of steatosis and intrahepatic invasion in patients undergoing liver resection for metastatic adenocarcinoma

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Introduction and Aim: To identify the association of clinicopathological factors with morbidity and survival in a cohort of patients undergoing liver resection for metastatic colorectal adenocarcinoma. **Methods:** Data were collected retrospectively on 90 consecutive patients undergoing a potentially curative liver resection for metastatic colorectal adenocarcinoma between 1995 and 2000. Intrahepatic spread was classified as vascular, bile duct, perineural and lymphatic invasion. NASH scores were according to the 'Brunt' system. Primary endpoints were mean survival time and time to normalisation of bilirubin and ALT following resection. Analysis was performed using the log-rank and the Kruskal-Wallis tests. **Results:** In the 90 liver resections, there were two perioperative deaths. The mean (SE) overall survival time in months for patients with single metastases was greater than for patients with multiple metastases 35.9 (5.1) vs. 27.4 (3.8); patients with vascular invasion had shorter survival times, 35.6 (3.5) vs. 40.3 (7.6); as did patients with lymphatic invasion 29.8 (4.2) vs. 42.1 (4.5). These trends were not statistically significant. The mean (SE) time to normalisation of ALT in patients with no evidence of NASH or mild disease was 18.2 (2.9) days. In patients with moderate and severe NASH, times to normalisation of ALT were significantly raised: 24.2 (4.4) days and 58.9 (14.4) days respectively (p=0.01). **Conclusion:** The presence of moderate/severe NASH is associated with an increased risk of peri-operative morbidity as measured by liver enzymes after liver resection. A non-statistically significant reduction in survival is demonstrated in patients with lymphatic and vascular invasion and in liver resection for multiple metastases.

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The septin-binding protein anillin is over-expressed in diverse human tumours

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Anillin is an actin binding protein that can form associations with septins, and has been shown to be a component of the cytokinetic ring. We assessed the anillin expression in 7579 human tissue samples and cell lines by DNA microarray analysis. Anillin is expressed ubiquitously but with variable levels of expression, being highest in the CNS. The median level of anillin mRNA expression was consistently higher in tumours than normal tissues (median fold increase 2.58, 95% confidence intervals 2.19 to 5.68, $p<0.0001$) except in CNS were anillin mRNA levels were lower in tumours. We developed a sensitive RT-PCR strategy to demonstrate that anillin mRNA is expressed in cell lines, and in cDNA panels derived from fetal and adult tissues, thus validating the microarray data. We compared anillin with Ki67 mRNA expression and found a significant linear relationship between anillin and Ki67 mRNA expression (Spearmann $r \sim 0.65$ to 0.75, $p<0.0001$), except in the CNS where $r = -0.11$ ($p<0.0001$). Anillin mRNA expression was analysed during tumour progression in breast, ovarian, kidney, colorectal, hepatic, lung, endometrial and pancreatic tumours and in all tissues there was progressive increase in anillin mRNA expression from normal to benign to malignant to metastatic disease. Anillin is thus over-expressed in diverse common human tumours and may have potential as a novel tissue biomarker. Finally we employed anti-anillin sera and found nuclear anillin immunoreactivity to be widespread in normal tissues, often not correlating with proliferative compartments. These data provide insight into the existence of non-proliferation associated activities of anillin and roles for anillin in interphase nuclei.

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Using Telepathology for Rapid Assessment of Fine Needle Aspirates in the One-stop Breast Clinic

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The aim of this study was to compare the diagnostic accuracy of a telepathology system with conventional cytological assessment of fine needle aspirates from a one-stop breast clinic.
The aspirates were performed by the radiologists at the Breast Screening Unit. The slides were prepared and stained using Diff-Quik by a biomedical scientist (BMS). The slides were screened by the BMS and live images, captured by a digital camera, were transmitted in real time via a dedicated link to the cytology department at another site. The Cytopathologist at the receiving station viewed the images in real time and gave the provisional diagnosis to the clinician. The Cytopathologist and BMS communicated by a telephonic link for field selection, appropriate lens selection and focusing. All slides were later reviewed in the department and a final report issued.
A total of 124 cases were included and concordance between telepathology diagnosis, final cytological diagnosis and histology were compared using NHSBSP guidelines.
The accuracy of remote diagnosis was comparable to conventional cytology. The complete sensitivity was 96% and there were no false positives or false negatives.
We conclude that telepathology is a potential alternative to an on- site cytopathologist in the rapid assessment of FNAs in the breast clinic.

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Grade, BCL2 Gene Abnormality and Expression, and Clinical Relevance in Follicular Lymphoma

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There is accumulating evidence that subdivision of follicular lymphoma (FL) on the basis of presence or absence of t(14;18)(q32;q21) may more accurately identify biologically relevant subtypes than a system based purely on grade. To further investigate the biological spectrum of FL the clinical, pathological and genetic features of 100 cases were studied. Comparisons were made between groupings arrived at on the basis of grade, or on the presence or absence of BCL2 gene abnormalities, the latter further stratified according to bcl-2 protein expression. FL grade 3 only differed from FLs grades 1 and 2 in having a lower incidence of t(14;18)(q32;q21), or equivalent abnormality, and lower bcl-2 protein expression ($p<0.05$ and $p<0.01$ respectively). A similar number of differences were seen when FLs harbouring abnormalities of the BCL2 gene were compared with cases displaying a germline BCL2 configuration. The former were significantly more likely to express bcl-2 protein ($p<0.001$) and be of advanced stage at presentation ($p<0.05$). The greatest contrast was seen when FLs with an abnormal BCL2 gene configuration and showing expression of bcl-2 protein were compared with bcl-2 negative FLs with germline BCL2. The latter were significantly more likely to be of high cytological grade ($p<0.01$), harbour abnormalities of 3q27 ($p<0.05$), be of early stage at presentation ($p<0.01$) and have a lower mean number of chromosomal abnormalities per case for grade 3 lesions ($p<0.05$). These results provide further evidence that grade 3 FL is not a uniform entity but that there is a distinct subgroup of bcl-2 negative FLs with germline BCL2 gene configuration, characterised by a predominance of grade 3 lesions, presentation with localised disease and a high incidence of translocations involving 3q27.

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Follicular Lymphomas Lacking t(14;18)(q32;q21) Constitute a Clinicopathological Subtype

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It has been proposed that primary cutaneous follicular lymphoma (PCFL) is a clinicopathological entity, distinct from nodal follicular lymphoma (FL), categorized by an absence of t(14;18), and an excellent prognosis. However, cases of t(14;18)(q32;q21)-positive PCFL, and examples of t(14;18)(q32;q21)-negative FL arising at nodal and other extranodal sites, are well documented. This raises the possibility that the presence or absence of the translocation may be a more meaningful way to identify biologically distinct subtypes of FL. Moreover, most studies of PCFL have only included cases presenting with stage 1 disease. This makes it difficult to meaningfully compare outcomes with nodal FL, the latter usually being of advanced stage at presentation. We therefore investigated a cohort of 49 stage 1 FL (20 cases of nodal FL, 15 cases of PCFL and 14 cases of stage 1 FL arising at non-cutaneous extranodal sites) stratified according to the presence or absence of t(14;18)(q32;q21) using classic cytogenetics, PCR (mbr, mcr and icr primers) and interphase FISH. Compared with t(14;18)(q32;q21)-positive cases, FL lacking the translocation more frequently displayed grade 3 morphology, were less likely to express CD10 or bcl-2 ($p<0.01$) and made up a significantly greater proportion of cases arising at extranodal sites ($p<0.001$). Although the frequency of relapse was similar in both groups, there was a more favourable overall survival in the t(14;18)(q32;q21)-negative cohort ($p<0.01$). These results support the concept of a subtype of FL lacking t(14;18)(q32;q21), and characterized by grade 3 morphology, low intensity bcl-2 expression, a predilection for extranodal sites and more favourable outcome than t(14;18)(q32;q21)-positive FL.

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Homicidal Commotio Cordis – A Three-case Report

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Commotio cordis is a recognised cause of sudden death in otherwise healthy individuals, and results from an impulse-like blow over the cardiac shadow. If this coincides with the upstroke of the T-wave on ECG it can produce ventricular fibrillation and sudden death. It is best documented in sporting situations, most commonly baseball, although it has been reported in a wide range of other circumstances. We report three cases where it seems most likely that this mechanism has been involved in fatal assaults. The individuals involved had evidence of an impact to the chest, but no injuries that would, in themselves, appear to account for their demise. These cases serve to illustrate a relatively rare cause of sudden death which has a well-researched scientific basis but may be encountered by any pathologist undertaking medico-legal autopsies in a range of situations, and which may provide an explanation for deaths which may otherwise remain obscure.

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Abstract Withdrawn

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Malignant Mesothelioma Of The Peritoneum In A Woman

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A 76 years old female presented with an umbilical lump. The histology showed a tumour in keeping with a metastasis from a malignant mesothelioma (MM). There was no history of asbestos exposure. Serum CA125, CA19-9 and CEA were not elevated. Primary peritoneal mesothelioma was diagnosed.
The patient later complained of abdominal pain and nausea, and died after four weeks. A coroner's autopsy was performed. The autopsy finding was diffuse tumour deposits in the peritoneal cavity. The thoracic cavity did not show any plaques or deposits. Both lungs showed pulmonary oedema on slicing. The histology of the tumour deposits was identical to the initial tumour biopsy. The lung histology showed bronchopneumaonia. No asbestos bodies were found. The cause of death was 1a bronchopneumonia , 2 carcinomatosis due to disseminated peritoneal malignant mesothelioma (PMM).
In contrast and unlike pleural mesotheliomas, peritoneal mesotheliomas in women do not appear to be closely related to asbestos exposure. This case probably falls in this group of PMM with no history of exposure to asbestos. We conclude that peritoneal mesotheliomas can be diagnosed without a history of asbestos exposure, and every effort should be made to exclude a papillary serous ovarian tumour by specific antibodies.

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Brain Embolism To The Lung After Gunshot Wound To The head

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Introduction: The occurrence of embolisation of cerebral tissue to the lungs is a rare event related to severe heard trauma. Only two cases of massive pulmonary embolisation with cerebral tissue have been reported following gunshot wound. The present case is a further report of this phenomenon with immuno-histochemistry confirmation. The history and post mortem examination findings of the same are presented.
History: The deceased was a 42 year old male with history of depression and cannabis abuse. He was found dead in his home and a jammed pistol was found near the body.
Post-mortem examination findings: External examination revealed an abrasion over the temporal area on the right side. On internal examination there was linear crack fracture in the right frontal bone, fragmentation of the left side of C2 and C3 vertebrae and a bullet underneath the scalp of the left parietal bone. Respiratory system examination revealed pale material embedded in the small pulmonary vessels. This was confirmed to be cerebral tissue within the pulmonary arterial branches by histological examination and immuno-histochemistry.
Conclusion: Presence of the cerebral tissue in the vessels of pulmonary parenchyma, both macroscopically and microscopically, must be carefully looked for in cases of severe head trauma

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Spontaneous Right Ventricular Rupture: An Unusual Complication Of Mediastinitis After Cardiac Surgery – A Case Presentation

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A 57 year old man had coronary artery bypass grafting for triple vessel disease. Postoperatively he developed sternal wound infection requiring sternal wound debridement and re-wiring. The wound failed to heal, necessitating further reconstructive surgery including sternotomy and muscle flaps. His blood pressure plummeted soon after extubation. During cardiopulmonary resuscitation the patient's chest started to expand and the drains filled up with fresh blood. Sternal wound was re-opened, which showed a large amount of pulsating arterial blood. The exact point of bleeding could not be identified and the patient died.

At post-mortem an open infected sternal wound was observed. Sternum had been removed. Underneath the muscle flaps, the anterior surface of the right ventricle was directly visible with a small rupture associated with friable surrounding ventricular muscle and pericarditis. Adhesions between the anterior surface of the right ventricle and under-surface of the chest wall were also present. No apparent graft rupture could be identified.

We concluded that the sternal wound infection had extended on to the surface of the heart leading to pericarditis and myocarditis, making the infected friable myocardium susceptible to rupture and haemorrhage which proved to be the terminal event in this case.

This is an unusual but known complication of cardiac surgery. A literature review suggests several reported cases including some, where early identification resulted in successful repair.

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A Post Mortem Case Of Community Acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA)

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A 28-year-old female otherwise healthy was admitted to hospital with a 3-day history of flu-like illness. The clinical diagnosis was lobar pneumonia. Few hours after admission the patient died in the intensive therapy unit.
The post mortem revealed both lungs that were firm, consolidated showing changes of haemorrhagic pneumonia. Lung tissue and blood sample was taken. The lung histology showed features of haemorrhagic pneumonia. Polymerase chain reaction studies for adenoviruses and flu virus were negative. The lung tissue culture and the ante-mortem bronchial washings, both grew a strain of Staphylococcus aureus. After extended genetic characterization the isolate resembled closely a CA-MRSA clone found in Europe.
MRSA has been until recently regarded a hospital-associated pathogen (H-MRSA), but has been increasingly identified as a cause of community-onset infections. CA-MRSA has several distinguishing features from H-MRSA including: Presence of the SCCmec element and presence of gene encoding the Pantone-Valentine leukocidin toxin. The isolate from this case possessed both these features. CA-MRSA is known to cause fatal pneumonia in young immuno-competent population.
Pathologists must think about this entity, especially in 'young healthy individuals', when performing post mortems showing changes of haemorrhagic pneumonia grossly or histologically.

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Case Report: Metachronous and Synchronous Lung Tumours: Five Malignant Lung Pathologies in One Patient over 7 Years.

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Patients treated surgically for lung carcinoma have a risk of having a second primary tumour. We present the case of a 70 year old man who previously had a left upper lobectomy for non-small cell lung carcinoma, subsequently developed small cell carcinoma of the right lower lobe, which was successfully treated and finally had a right upper lobectomy, which revealed three synchronous lung tumours of different histological types. We were unable to find a previous report of a total of five separate lung malignancies with a combination of metachronous and synchronous tumours. This case demonstrates the importance of screening following the diagnosis and treatment of lung carcinoma.

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The diagnostic value and cost-effectiveness of bronchial brushings in the diagnosis of bronchial malignancy

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A retrospective study to ascertain the diagnostic value of bronchial brushings, when washings and biopsy were also available, and to assess associated costs and workload implications.

We identified 104 cases where bronchial washings, brushings and biopsy had been obtained at bronchoscopy, and ascertained their relative contribution to the report. We observed the time spent by various grades of staff in the bronchoscopy unit and the laboratory in collecting, processing and reporting them, and the consumables used. As a measure of cost-effectiveness, incremental cost effectiveness ratios were calculated.

Forceps biopsy was found to be the most diagnostically useful specimen, with 64/75 malignancies having a positive biopsy. 11 cases were diagnosed by cytology alone. In two cases, over the two-year period, brushings provided the only positive result. There was a significant difference in the number of cases where cytology provided corroborative evidence to the histology, with washings more often supporting the biopsy than brushings.

The approximate cost to the laboratory of each additional diagnostic test was found to be £425. The approximate cost to the hospital was £1380. The additional technical time for each diagnostic test was 21 hours, and 4-5 hours of pathologist time.

Given the wide variation in bronchoscopic and laboratory practice between different hospitals, it may be appropriate for each laboratory/bronchoscopy unit to conduct its own audit of the cost-effectiveness of this and other tests.

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Pathological Findings in Sudden Cardiac Death

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Sudden cardiac death results from an acute ischaemic event or a ventricular arrhythmia arising in an inflamed, scarred, hypertrophied or genetically abnormal heart. To determine the relative contributions of these causes made a prospective study of all cases of sudden cardiac death in adults of any age over a one year period.

Three hundred and twenty one cases of out-of-hospital sudden cardiac death occurring within one hour of the onset of symptoms were studied. Each case was graded according to the certainity of the cause of death. Grade 1 cases were the certain result of ischaemia and had clear evidence of coronary artery thrombosis or recent myocardial infarction.. Grade 2 cases had evidence of healed myocardial infarction and at least one coronary artery < 1mm in diameter. Grade 3 cases had coronary artery narrowing alone while Grade 4 had cardiac hypertrophy only. Grade 5 was unexplained cardiac death (SADS). In 107 cases (33%) there was coronary thrombosis or acute infarction. In 163 further cases there was coronary artery narrowing. 107 (33%) had an associated myocardial scar (Grade 2) whilst 56 (17%) did not (Grade 3). In 45 cases (14%) non ischaemic heart disease was present (Grade 4) and in 5 there was no demonstrable cardiac pathology (Grade 5, SADS)

The 33% incidence of acute ischaemic in sudden death is lower that in two studies from the 1990s (47% and 52%). Whether this is the result of current limitations in autopsy practice or a real change in the pattern of disease is uncertain. Our incidence of SADS is lower than that found in a recent national study, but one that set a upper age of 65 years. Sudden death due to unexplained cardiac hypertrophy is important and has been underinvestigated.

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Diagnostic Value Of Bronchial Washings And Brushings In The Diagnosis Of Bronchial Malignancy

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We examined all bronchoscopy cases over a 6 month period and correlated the results of bronchial washings, bronchial brushings and forceps biopsy. We identified 140 cases, of which 44 were positive for malignancy. Of these 44 cases the forceps biopsy was the most useful diagnostic tool with 41/44 (93.1%) having a positive biopsy. Only three cases were diagnosed on cytology alone, two a combination of positive washings and brushings, one on brushings alone. No cases were diagnosed on bronchial washings alone. Our study supports the Royal College of Pathologists' recommendation to omit bronchial washings if a tumour is visible[1]. We may even suggest that bronchial brushings may also be omitted if a tumour is visible, as biopsy alone detected over 93% of cases. In this study clinical suspicion was sufficiently high that the three cases which relied on cytology would not have been missed.

1.http://www.rcpath.org.uk/filecollection.asp?file=DRAFTHistoOfLimitedOrNoClinicalValue2ndEdition.pdf Accessed 29/3/05
2.British Thoracic Society guidelines on diagnostic flexible bronchoscopy. Thorax 2001, Suppl I:i16-i17

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Dissecting Splenic Artery Aneurysm Due To Mediolytic Arteriopathy.

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Aim: To describe a rare case of segmental mediolytic arteriopathy of splenic artery presenting as haemoperitoneum.
Case Report: A 53 year old female presented with sudden onset abdominal pain, falling blood pressure, tachycardia and a weak pulse. She was a known hypertensive on treatment with no other significant past medical history. Emergency laparotomy showed haemoperitoneum (2000ml) and a mass was felt in the region of tail of the pancreas; this was clinically thought to be either an inflammatory mass or a splenic artery aneurysm. Distal pancreatectomy and splenectomy were performed.
Macroscopically, there was a blood clot around the splenic artery at the splenic hilum. The spleen and pancreas were normal.
Histology of the splenic artery showed focal intimal thickening, segmental mucinous medial degenerative changes with occasional microcyst formation and, haemorrhage into the media. The latter was seen focally within the media and, in other areas, was continuous and consistent with medial dissection. Loss of entire thickness of the wall with associated clot formation and dense neutrophilic reactive infiltrate was seen focally in association with periarterial inflammation in the region of rupture; no granulomas, giant cells or evidence of vasculitic injury were seen. Aneurysmal dilatation of this vessel was also observed as a result of the degenerative medial changes.
Conclusion: We describe a rare cause of non-atheromatous, non-vasculitic and non-fatal rupture of a dissecting splenic artery aneurysm due to mediolytic arteriopathy. It is important to recognise this condition because multiple arteries may be involved and a further investigation of the patient is mandatory.

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Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia. A Case Associated With UIP-like Fibrosis

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A 59 year old man presented with breathlessness and wheeze. CT scanning revealed multiple nodules throughout both lungs, interstitial markings and mediastinal lymphadenopathy. A subsequent left lower lobe wedge resection showed 5 peripheral-type spindle cell carcinoid tumours, 29 carcinoid tumourlets, organising pneumonia and UIP-like interstitial inflammation.
Multiple carcinoid tumours and tumourlets, plus the presentation, suggests a diagnosis of Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH), an exceedingly rare condition with only 12 cases reported.
Existing criteria for DIPNECH preclude concurrent inflammation/fibrosis which could, in theory, induce pulmonary neuroendocrine cell (PNC) hyperplasia. Tumourlets/PNC hyperplasia may be associated with lung inflammation, but no publications record a link between this combination and UIP or carcinoid tumours.
Peripheral carcinoid tumour with multiple tumourlets may occur without clinical features of DIPNECH and without pulmonary inflammation/fibrosis. The relationship between such cases and DIPNECH is uncertain.
It is most likely that this case is an example of DIPNECH and that the UIP-like pattern of interstitial disease is incidental. However, given that PNC hyperplasia is thought to cause fibroplasia (accounting for the small airway obstruction in DIPNECH), it is conceivable that the UIP-like change could be caused by some factor secreted by the PNCs in this patient's lung.

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Cardiac Fibromas – A possible significant 9q22 chromosomal breakpoint.

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Introduction: Cardiac fibromas are rare tumours, and only one case has previously been subjected to cytogenetic analysis.
Case Report: A fourteen-month old girl presented with recurrent ventricular tachycardia and was diagnosed with a ventricular septal tumour. Histologically, the tumour was composed of bland spindle cells devoid of mitotic activity and set in a collagenous matrix. Focal areas of calcification were noted. The appearances were those of a cardiac fibroma.
Cytogenetic Analysis: The cells from the tumour culture contained an abnormal clone with 46chromosomes and a 3-way translocation, 46,XX,t(1;9;5)(q42;q22;q22). Other abnormalities included a single tetraploid cell with the same translocation, and an isochromosome 6p.
Comment: A case of cardiac fibroma has been reported previously which showed translocation t(1;9)(q32;q22). The common chromosomal breakpoint for the two cases is 9q22. The 9q22 breakpoint may therefore be significant in the development of cardiac fibromas. This case emphasizes the importance of receiving unfixed tissue from rare tumours to allow karyotyping and identification of possible significant translocations.

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TURP Specimen Sampling And The Detection Of Prostatic Adenocarcinoma

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There is variation between our local protocol for sampling transurethral resection of prostate (TURP) specimens and the Royal College of Pathologists (RCPath) guidelines. Compliance with local standards was audited and the additional workload which would result from adherence with the RCPath guidelines was then determined. Sampling of these specimens should be adequate to diagnose incidental prostatic adenocarcinoma. The ability of our laboratory to do this was also appraised.

The first audit identified 50 consecutive patients who had undergone TURP. The request forms and histopathology reports were reviewed, and the sampling of these specimens was compared with local and RCPath guidelines. Subsequently 91 cases were reviewed where a new diagnosis of adenocarcinoma had been made on TURP specimens and the number of blocks needed for diagnosis was established.
The major deviation from local standards was not embedding all the material in patients aged 65 years and under. Following RCPath standards would have produced an additional 979 blocks over 12 months. Diagnoses of adenocarcinoma were made on the first block in 57% of cases, and by block six in 98%.
These results indicate that our protocols are adequate to diagnose prostatic adenocarcinoma in TURP specimens, and that processing over six blocks may be unnecessary.

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Paediatric Percutaneous Renal Biopsy Pathology: A Review Of 1,271 Cases From a Single Centre

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Introduction: Percutaneous renal biopsy remains the 'gold standard' diagnostic and prognostic investigation for a range of renal conditions. In childhood the indications and common pathologies are different to those in adult practice. This study reviews a large series of paediatric renal biopsies from one centre.
Methods: A retrospective review was carried out of the pathological findings from all diagnostic paediatric renal biopsies carried out for the clinical indication of renal disease (excluding renal tumours) during a 15 year period (1988-2002).
Results: There were 1,271 cases in total. The most frequent biopsy pathological diagnoses were minimal change nephropathy (19%), idiopathic focal segmental glomerulosclerosis (9%), transplant rejection (8%), lupus nephritis (5%), Henoch-Schonlein nephritis (5%), IgA nephropathy (4%), Alport disease (3%), chronic transplant rejection (3%), post-infectious glomerulonephritis (3%), diffuse mesangial proliferative glomerulonephritis (3%), interstitial nephritis (3%) and membranoproliferative glomerulonephritis (3%). Although uncommon, (3% of total), also included were cases of specific congenital paediatric renal diseases such as Finnish type congenital nephritic syndrome, diffuse mesangial sclerosis and autosomal recessive polycystic kidney disease.
Conclusion: Renal biopsy in childhood provides important clinical information. In this large series, minimal change nephropathy and primary focal segmental glomerulosclerosis (FSGS) account for about one third of cases, however, a wide spectrum of renal disease may be encountered in children including congenital and acquired conditions uncommonly encountered in adult practice.

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Post-transplant Proteinuria Due to De Novo Membranous Nephropathy in Childhood

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Introduction: Post-transplant significant proteinuria occurs in around 10-15% of renal transplant recipients. In the majority, the cause is either chronic allograft nephropathy or recurrence of native glomerulonephritis. In a minority, de-novo post-transplant membranous nephropathy (DNPMN) is identified on biopsy. We present a case of DNPMN in childhood and review the pathology, pathogenesis and clinical implications of this condition.
Case Report: The patient underwent renal transplantation aged nine years for severe congenital obstructive uropathy. Seven years later his serum creatinine concentration began to rise and biopsy demonstrated moderate chronic allograft nephropathy and borderline rejection. During the following six-months his creatinine continued to rise and he developed proteinuria with hypoalbuminemia. A repeat biopsy confirmed chronic allograft nephropathy with chronic allograft glomerulopathy, calcineurin inhibitor effect and focal 'spike' formation. Electron microscopy demonstrated widespread glomerular capillary wall subepithelial deposits indicating membranous nephropathy.
Discussion: Pooled data from 3,879 transplants reports that 10-15% will develop persistent proteinuria at an average 24 months post-transplantation, which is associated with a relative risk of graft loss of 3.5. Pooled Data from >4,000 post-Tx renal biopsies reports the prevalence of PTDMN to be at least 1.5%, (greater when electron microscopy is widely used), being the cause of about 5-10% of cases with post-transplant proteinuria, and associated with subsequent graft loss in more than 50% of patients.
Conclusion: It is important that post-transplant renal biopsies in cases with proteinuria be assessed in a similar way to native biopsies, using accessory techniques such as immunohistochemistry and electron microscopy.

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Clinicopathological Correlation In Paediatric Lupus Nephritis: Relationship To Revised Histopathological Classification

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Introduction: The International Society of Nephrology and Renal Pathology Society Working Group recently revised the histopathological classification of lupus nephritis (LN) but the clinical implications of the new classification in paediatric LN remain uncertain.
Methods: This study evaluates clinical outcomes of children with lupus by retrospectively reclassifying percutaneous renal biopsies according to the revised criteria.
Results: During the last decade, 39 children aged 3.3-18.0 (median 13) years underwent 49 percutaneous renal biopsies at 0.2-7.8 (median 1.0) years from SLE diagnosis at our centre, representing an incidence of LN in our paediatric SLE population of 66%. With the revised classification, there were 6 cases of overlap between the classes giving total number of cases of Class I (1 or 2%), II (7 or 13%), III (8 or 15%), IV (28 or 51%), V (11 or 20%) and no cases of WHO Class VI. There were two cases of LN which showed features of both WHO Class III and V LN and four cases with overlap of WHO Class IV and V LN. Patients were followed up for 0.8-14.9 (median 5.0) years and the overall survival rate was 94%. The median estimated GFRs were 112.7, 116.4, 93.6, 81.0 and 118.5 mls/min/1.73m^2 for LN Classes I-V respectively. The three most severe cases of chronic renal failure with eGFRs <25 mls/min/1.73m^2 were in patients with diffuse global sclerosing lupus nephritis (WHO Class IV-G(C)).
Conclusion: In childhood, lupus nephritis occurs in around two-thirds of those with SLE, of whom about half will have diffuse (Class IV) LN, which carries the worst prognosis, in particular with diffuse sclerosing LN. The revised LN classification appears to provide useful clinical information in paediatric LN biopsies.

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Evaluation of the Role of Electron Microscopy in Native Renal Biopsy Interpretation

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In the histopathological evaluation of renal biopsies, electron microscopy (EM) may be performed alongside light microscopy and immunohistochemistry / immunofluorescence analysis. There is national variation regarding its use in renal biopsy interpretation. Our aim was to assess the value of EM in the histological assessment of renal biopsies in two hospitals. Retrospective evaluation of native renal biopsy reports from January 2000-June 2001 was carried out. The contribution of EM to the final diagnosis after light microscopy and immunohistology were performed was evaluated. In one hospital, the pathologist issued a preliminary diagnosis while awaiting the EM result, in contrast to the other hospital where a diagnosis was only issued once the EM result was available. The reports from the first hospital were also assessed in terms of whether the EM result altered the preliminary diagnosis. EM was found to be essential in 28-30% and contributory to the final diagnosis in 44-54%. 21% of preliminary diagnoses were changed after the EM result was received. EM remains a valuable tool in renal biopsy interpretation and, we believe, should be performed routinely in all cases. Where this is not possible, tissue should at least be retained for EM examination.

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Topoisomerase II-Alpha Expression Correlates with Gleason Score in Prostate Carcinoma

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Background: Topoisomerase II-Alpha is a cell proliferation marker. It is also the target of the drug etoposide, which is an active agent in the combined chemotherapy of hormone-insensitive prostate carcinoma.
Methods: We compared the immunohistochemical expression of Topoisomerase II-Alpha antibody in 4 groups - benign prostatic hyperplasia (n=41), prostate carcinoma Gleason Score 6 (n=28), prostate carcinoma Gleason Score 7-8 (n=35), and prostate carcinoma Gleason Score 9-10 (n=37) using tissue microarrays. We also compared subgroups of prostate carcinomas Gleason Score 8-10 under the categories of hormone resistant (n=14) and hormone responsive (n=34). We used the Mann- Whitney U test to determine whether there was a statistically significant difference between the Topoisomerase II-Alpha indices in each group.
Results: There was a highly statistically significant difference between the group's benign prostatic hyperplasia and prostate carcinoma Gleason Scores 9-10 (P<0.0001). There was a statistically significant difference between the group's benign prostatic hyperplasia and prostate carcinoma Gleason Scores 7-8 (P=0.04). The difference in the prostate carcinoma cohorts hormone resistant and responsive approached statistical significance (p=0.052).
Conclusion: Topoisomerase II-Alpha expression correlated with the known prognostic marker of Gleason Score however there was no significant increased expression in Gleason Score 6 prostate carcinoma compared with benign prostatic hyperplasia.

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Adenocarcinoma Of The Urinary Bladder Associated With Schistosomiasis Immunohistochemical Profile

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BACKGROUND & METHODS
A series of 133 patients with primary non-urachal adenocarcinoma of the urinary bladder diagnosed and treated at the Cairo NCI were reviewed. Tumors were classified as intestinal colonic (102) and non-intestinal urothelial (31) types. LSAB method using antibodies to cytokeratins7&20, p53 protein, HER-2/Neu &cathepsin D.
RESULTS
Associated colonic metaplasia is present in 23% of cases &24% of intestinal adenocarcinoma had areas which resembled villous or tubular colonic adenomas. Cytokeratin20 was expressed by 90%of intestinal and by 10%of urothelial adenocarcinomas. Cytokeratin7 was expressed by 87% of urothelial and 17%of intestinal- type tumors. Areas of cystitis glandularis and cystica of the bladder urothelium showed complete or partial loss of cytokeratin7 and intense expression of cytokeratin20. p53 over-accumulation was observed in 84%&48% of intestinal&urothelial adenocarcinomas, respectively. HER-2/Neu & Cathepsin D is weakly and focally expressed.
Conclusions
Primary intestinal type adenocarcinoma of the urinary bladder is morphologically and immunohistochemically identical to colonic adenocarcinomas. The frequent association of adenocarcinoma with colonic metaplasia and colonic type adenomas suggest a possible multi-step carcinogenic pathway similar to that observed in colorectal carcinomas.

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Polymorphs Within Renal Tubules And Urinary Infection In Acute Transplant Rejection-A Dilemma For The Pathologist

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Introduction: Polymorph collections within renal tubule lumens is conventionally associated with urinary tract infection/pyelonephritis. Renal transplant biopsies showing acute rejection sometimes have a few tubules containing lumenal polymorphs and this inevitably raises the dilemma of whether there may additionally be infection present.
Pathologists usually put a rider into their report to the effect that infection should be excluded 'just in case'. Our impression is that this feature does not usually indicate infection in this context. The aim of this study was to try and substantiate this impression.
Methods: A retrospective analysis of transplant biopsies showing acute rejection over a two year period was undertaken. Biopsy reports were scrutinized and any biopsies describing polymorphs in tubule lumens were extracted. The slides were reviewed to confirm this. The clinical notes were reviewed, and any urine culture evidence of infection around the time of biopsy noted. All cases had urine culture data available.
Results: Fourteen cases were identified, of which two showed urine culture positivity around the time of biopsy.The other cases were culture negative.There was no significant association between the presence of polymorphs and urine culture positivity.
Conclusions: Polymorphs in tubule lumens in acute rejection are not usually associated with urinary infection.

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Malakoplakia Of The Glans Penis

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Abstract:
Malakoplakia is an inflammatory condition usually affecting the genitourinary tract and gastrointestinal tract. In rare instances there is skin involvement. This is a report of a unique case of Malakoplakia occurring on the glans penis.

A 77 year old Caucasian male presented with tender erythematous lesions on the glans penis, which had been present for six years. He had long standing pruritus. He had been applying topical steroids to the penis resulting in skin atrophy. A punch biopsy of a nodular lesion of the glans penis was performed.

Histopathological examination revealed an infiltrate of histiocytes with fine eosinophilic cytoplasmic granules. (Von Hansemann Cells) admixed with lymphocytes and plasma cells. There were many scattered histiocytes with typical Michaelis –Gutmann bodies, which demonstrated positivity with periodic acid –Schiff's stain after diastase. These bodies stained with Von Kossa and Perls Prussian blue indicating the presence of calcium and iron. Gram stain demonstrated Gram –negative bacilli.
The patient was not immunocompromised but had six years of intermittent topical steroid treatment for pruritus of the glans penis. It is postulated that such local treatment may affect local macrophage function such that the glans penis was in effect immunocompromised thus leading to Malakoplakia.

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The Use Of Levels In Detecting Cancer In Prostate Biopsies

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Considerable variation exists in UK pathology departments, with no standardised guidelines for the number of levels needed to examine prostate needle core biopsies. A protocol was introduced in this department for examination of three initial levels and, if no cancer detected, three further levels. This study sought to determine the effects of the protocol on cancer detection, the minimum number of levels required and the value of clinical information in selecting which specimens to further examine.
All prostate needle biopsies over 12 months were assessed for pathology present in initial and further levels, and the level in which cancer first appeared. Of the 191 specimens included 73 cases showed cancer (38.2%). Cancer was first identifiable within levels 1 to 3 in 72 cases (98.6%) and in level 4 in one case (1.4%). In two cases of initially unilateral carcinoma further levels revealed bilateral disease. Age, PSA and clinical impression of malignancy were unhelpful in predicting cancer in biopsies received.
Routine examination at more than three levels occasionally reveals additional cancers, with potentially important consequences. We suggest a minimum of four levels to maximise cancer detection and that additional levels should not be limited to “suspicious” cases based on clinical information.

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A Study Of pT1 Bladder Carcinoma Highlighting Pitfalls In Diagnosis And The Benefit Of Sub-grouping Into Focal And Widely Invasive Disease

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Two pathologists with an interest in Urological pathology reviewed cases of pT1 urothelial carcinoma of bladder diagnosed in a single institution over 2 years. 46 cases were identified from the files. On review, it was felt that 18 cases had been incorrectly staged pathologically. In 15 cases, review suggested a pTa neoplasm. Common errors were related to misinterpretation of diathermy artefact or an inverted growth pattern. 3 cases showed foci of muscle invasion that had been overlooked originally, upgrading these tumours to pT2. Those cases with genuine lamina propria invasion were subdivided into those with minimal invasion (pT1a) and those with widespread invasion (pT1b). 11 low grade tumours were identified, 10 restaged as pTa and one as PT1a. 25 high grade tumours were identified, 5 pTa, 8 pT1a, 19 pT1b and 3 pT2. None of the patients with low-grade tumours or pTa / pT1a staging showed disease progression/died of their disease. Only patients who had high-grade tumours that were either pT1b or pT2 at diagnosis showed disease progression/died of their disease. We conclude that it is worthwhile sub-dividing pT1 bladder tumours into minimally invasive or widely invasive, as it appears to provide useful prognostic information.

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Membranous Nephropathy Associated With Tubulo-interstitial Nephritis And Anti-tubular Basement Membrane Antibodies

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We describe a rare case of membranous nephropathy associated with tubulo-interstitial nephritis and anti-tubular basement membrane antibodies in a 6 year old boy.
The boy presented with sudden onset haematuria, proteinuria, glycosuria, polydypsia and polyuria. Investigations demonstrated evidence of proximal renal tubular dysfunction (Fanconi syndrome).
The renal biopsy showed features of membranous nephropathy and tubulo-interstitial nephritis with moderate chronic tubulo-interstitial damage. Immunofluorescence studies demonstrated intense finely granular deposition of IgG and C3 on the glomerular capillary walls and on the tubular basement membranes. Moderate granular deposition of IgA and C4 was also identified on glomerular capillary walls.
Electron microscopy of the glomerular capillary basement membranes showed numerous subepithelial and intramembranous electron dense deposits, in keeping with membranous nephropathy.
This is an example of a rare association of membranous nephropathy with anti-tubular basement membrane antibodies and tubulo-interstitial nephritis presenting with tubular dysfunction. The pathogenetic mechanisms are unclear and genetic and environmental factors have been postulated to be of significance.

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Presentation of Fabry’s Disease as an incidental finding associated with renal oncocytoma

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A 58 year old woman with normal renal function underwent a laparoscopic left radical nephrectomy for a renal oncocytoma.

In the backround renal parenchyma, the glomeruli contained prominently vacuolated enlarged visceral epithelial cells. The histological appearances were suggestive of a storage disorder and electron microscopy was performed.

Electron microscopy showed features characteristic of Fabry’s disease. This is a rare metabolic disorder resulting from a point mutation at Xp22, causing a deficiency in alpha-galactosidase A. The disease is completely expressed in males, while females can be clinically asymptomatic.

This case represents an unusual presentation of a rare disease with important clinical and genetic implications.

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Tissue Microarray Immunohistochemistry and Cluster Analysis Confirms the Validity of Candidate Proteins in Prostate Cancer Identified by Proteomic Analysis of Urine

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Our proteomic analysis of urine from patients with and without prostate cancer (by gel electrophoresis and mass spectrometry) identified a number of proteins whose expression was lost in cancer including S100A6, S100A8, S100A11 and lactate dehydrogenase. Antibodies to these proteins were obtained and immunohistochemistry was performed on tissue microarrays containing samples of prostate cancer and non-neoplastic prostatic epithelium (benign prostatic hyperplasia and morphologically normal background epithelium from cancer cases). Staining was scored semi-quantitatively on triplicate cores. The data from all 4 proteins was analysed using cluster analysis and a visualisation tool (Cluster & Treeview, EisenLab, University of California, US; de Hoon, University of Tokyo). There was clear clustering at the primary bifurcation of the hierarchical tree into benign and malignant groups. This division gave a sensitivity of 96%, specificity 89%, predictive value of positive result 79% and predictive value of negative result 98%. This analysis shows that the proteomic analysis of small numbers of samples (6 with and 6 without cancer in this study) is a valid method of identifying candidate proteins in cancer, and confirms the validity of these specific candidates.

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An audit of the Histological reporting of Prostate Specimens in a District General Hospital

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Objective
To evaluate the histological reporting of prostatic core biopsies and chippings against guidelines set out in the National Minimum Datasets and examine the correlation between parameters used for diagnosis and clinical information provided including the PSA level.

Methods
All prostate reports between the 1/10/2003 and 30/9/2004 were retrieved using the SnoMed system and the departmental archives. The reports were compared with the National Minimum Dataset guidelines and the compliance for each parameter was determined. In addition Gleason grade was compared with the PSA level as provided by the clinicians.

Results
In total 568 reports were retrieved including 354 core biopsies and 169 chippings. A positive or negative diagnosis of adenocarcinoma was documented in all reports and all diagnoses of adenocarcinoma included a Gleason score. However 87% of reports for prostatic adenocarcinoma did not refer to the presence or absence of lymphovascular invasion. In addition no statement was made regarding peri-prostatic tumour spread in 81% of reports for adenocarcinoma. Other parameters which were poorly recorded include high grade PIN and inflammation (not stated in 69% and 62% of reports respectively). In addition a correlation between Gleason Grade and PSA level was demonstrated (Correlation coefficient + 0.41 and $P<0.001$).

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Correlation of Gleason Score of Needle Biopsies & Prostatectomies & Comparison of Grading Practices in a Pathology Network

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The Gleason grade of prostate cancer is an important factor in influencing therapeutic decisions and predicting prognosis. We correlated Gleason score (GS) in preoperative needle core biopsies with radical prostatectomy specimens. We also compared the grading practices of 13 consultants in our network.

57 patients with RP for a period of 1 year were included in the study. Definitive postprostatectomy GS was accurately predicted by pre-operative biopsy in 52.6% and undergraded by 1 in 28.1% of patients.

Although a fairly good correlation was recorded between GS obtained on biopsy and prostatectomy specimens, the problem of undergrading remains to be improved.

We also compared grading practices of pathologists reporting needle biopsies at Pathlinks. The total number of needle core biopsies during same year was 871 of which 419 (48.1%) were malignant. The average GS allocated by each pathologist varied from 6.5 to 7.7. A similar variation in GS was reported in audits from other pathology departments.

The GS were regrouped into an intermediate and a high score group and compared with a reported Canadian study.

The results demonstrated minor variations and acceptable uniformity of Gleason grading of prostate cancers.They provide a baseline for pathologists to check their consistency of Gleason grading.

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Microstaging of Carcinoma of the Prostate: Can Biopsy Findings Predict if a Tumour is Clinically Insignificant

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Some tumours detected by PSA screening are clinically insignificant (defined here as tumour volume ≤ 0.5ml). If these tumours could be identified then treatment would be not be appropriate. In theory accurate sampling of the gland by a biopsy protocol should allow assessment of tumour volume.

Analysis of the diagnostic biopsy in 151 consecutive prostate cancer patients having radical prostatectomy (RP) was carried out to determine how well biopsy findings can predict tumour size. All patients had previously had protocol biopsies performed by a radiologist with a special interest in the biopsy technique. Biopsy specimens had been processed for histology in separate cassettes; on average 15mm of core was examined from each sample. Number of positive cores, maximum length of adenocarcinoma in a core (MLA), and RP tumour volume was recorded.

There was a strong correlation between both the number of cores positive and the MLA with the tumour volume and stage. 29 cases had only a single core positive and an MLA of $\leq$4mm, of these 15 had clinically insignificant tumours giving a positive predictive value of 52% and a negative predictive value of 90% for this threshold.

With consistent biopsy technique and meticulous histological processing biopsy findings have the potential to accurately predict tumour size.

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Expression And Subcellular Localization Of Maspin In Human Ovarian Epithelial Neoplasms: Correlation With Clinicopathologic Feature

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Background: Maspin is a non inhibitory member of the serpin family that is down-regulated in breast and prostate cancer, but over-expressed in pancreatic carcinoma. However, there were very few published data regarding the role of maspin in ovarian carcinoma. The aim: of the present study was to evaluate maspin expression in ovarian epithelial neoplasms and correlate its expression with some clinicopathologic parameters. Material & Methods: Seventy eight paraffin embedded ovarian specimens from patients with ovarian epithelial neoplasms comprised the material of this study. They included 18 benign, 14 low malignant potential (LMP) and 46 malignant epithelial ovarian neoplasms, in addition to seven specimens from normal ovarian tissues as a control. Results: Immunohistochemical study of maspin expression using streptavidin biotin immunoperoxidase method revealed that, normal ovarian surface epithelium did not express maspin as well as benign serous and mucinous ovarian epithelial neoplasm. However, all benign Brenner ovarian tumours were maspin positive. On the other hand, 57.14% of LMP tumours showed weak maspin expression and 63% of malignant ovarian epithelial tumours were maspin positive but 39.1% showed maspin over expression. The two malignant Brenner tumours studied were maspin negative. There was a trend for maspin expression with high grade, high stage, bilateral tumours and tumours with metastasis. Tumours that showed maspin over-expression showed higher mitotic index (MI) ($P = 0.02$). Invasive cancers were more likely to have predominantly cytoplasmic staining compared to LMP tumours.
Conclusion: Maspin was found to be expressed in a substantial proportion of ovarian tumours with poor prognostic parameters. These results may offer new insights regarding the role of maspin in ovarian cancer that may also impact diagnosis and treatment strategies. Moreover, variation in maspin expression between Brenner tumour and other epithelial surface ovarian tumour may indicate that the different histological types of ovarian epithelial tumours probably represent distinct disease entities and involve different molecular pathways.
Key words: maspin, epithelial ovarian neoplasms, immunohistochemistry

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Strategy for Defining Biologically Relevant Levels of p53 Protein Expression in Endometrial Adenocarcinoma

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The p53 labelling index has been proposed as a prognostic factor in endometrial adenocarcinoma. The p53 labelling index may vary with antibody concentration employed. We stained a series of uterine endometrioid adenocarcinomas with the anti-p53 antibody D07 at varying antibody concentrations. Six endometrioid adenocarcinomas of the uterus were stained with D07 antibody at varying concentrations. A single case of uterine serous carcinoma (USC) and a case of proliferative endometrium were also studied. Cases were scored on a scale of 0 (negative) to 6 (>95% nuclei positive). The USC exhibited nuclear positivity of >95% of tumour cells at all antibody dilutions. The case of proliferative endometrium exhibited nuclear positivity of >95% of epithelial cells at all dilutions up to 1 in 200. Greater than 50% of epithelial cells were positive at all dilutions up to 1 in 800. The proportion of positive tumour cell nuclei in the endometrioid adenocarcinomas varied markedly with the antibody concentration employed. By simply varying the concentration of antibody the p53 labelling index can be altered markedly. The implication is that the p53 labelling index is not reliable as a prognostic factor in endometrioid adenocarcinoma of the uterus and by extrapolation in other neoplasms. Comparisons between studies are not valid since different antibody concentrations have been employed.

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Difficulties in the Histopathological Diagnosis of Tubal Complete and Partial Hydatidiform Moles

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Introduction: Anecdotal experience and previous published reports suggest that a higher proportion of ectopic pregnancies are initially over-diagnosed as hydatidiform mole (HM). We review our experience with such cases and describe the histopathological features which may aid differential diagnosis.
Methods: The histopathology department of the trophoblastic disease unit was searched to identify cases with a referral diagnosis of HM in tubal ectopic pregnancies and the clinicopathological findings reviewed.
Results: During the study period (1986 to 2004 inclusive) there were 132 cases referred with a diagnosis of possible tubal HM. The final review diagnosis was partial mole in 2, complete mole in 5, and HM (NOS) in 1. Overall, 8 (6%) had a final diagnosis of HM, none of which developed persistent gestational trophoblastic disease. The main histopathological feature causing apparent difficulty in differential diagnosis was the common presence of florid extravillus trophoblast proliferation noted in tubal ectopic pregnancies with local trophoblastic 'invasion'. The histopathological features required for the diagnosis of HM in ectopic pregnancies are identical to those in uterine material, requiring definite abnormal, non-polar trophoblast proliferation and the other specific stromal and trophoblastic features as previously described.
Conclusion: Although rare, both complete and partial molar pregnancies may complicate tubal pregnancies, but florid extravillus trophoblast proliferation and early immature intermediate villi in non-molar tubal pregnancies may be overinterpreted as molar change.

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Pathological-Ultrasound Correlation of Routine Sonographic Examination in the Diagnosis of Hydatidiform Mole: Experience of >1,000 cases From a Regional Centre

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Introduction: Complete (CHM) and Partial (PHM) hydatidiform mole, are the commonest types of gestational trophoblastic neoplasia. Both usually present clinically as pregnancy failure, with the diagnosis suggested following routine histopathological examination of products of conception. This study examines the accuracy of pre-evacuation ultrasound examination compared to the histopathological 'gold-standard' in diagnosing CHM and PHM.
Methods: Retrospective search of consecutive cases referred to the Trophoblastic Disease Unit, Charing Cross Hospital, London, in whom pre-evacuation ultrasound findings were available. Basic demographic data, ultrasound features and final review pathological diagnosis were collected and cases anonymised.
Results: There were 1,053 cases evaluated, median maternal age 31 (range 15-54) years, median gestational age 10 (range 5-27) weeks. 859 (82%) referred cases had a review diagnosis of mole, including 253 (29%) CHM and 606 (71%) PHM. Pre-evacuation ultrasound suggested molar change in 378 (44%) of molar pregnancies, including 200 (79%) of the CHM and 178 (29%) of the PHM ($z=13.4$, $P<0.0001$). 51 (12%) of the 429 cases in whom ultrasound suggested molar change, had a final diagnosis of non-molar miscarriage.
Conclusion: Pre-evacuation ultrasound examination can help identify molar pregnancies but routine histopathological examination of evacuated products remains the gold standard. Ultrasound examination can detect the majority (around 80%) of CHM but only a minority (around 30%) of PHM, both of which require detection and hCG surveillance since both are associated with significant increased risk of development of persistent gestational trophoblastic neoplasia.

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Role of Central Review for the Histopathological Diagnosis of Complete and Partial Hydatidiform Moles in Southern England

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Introduction: In the UK, there is a well-established programme for the diagnosis, management and follow-up of pregnancies complicated by complete (CHM) and partial (PHM) hydatidiform moles, via two Regional centers in England. All suspected cases of PHM, and more recently, CHM are sent for central pathological review.
Methods: We searched the histopathology database of the Trophoblastic Disease Unit, Charing Cross Hospital, London, to identify registered 'definite' cases (rather than direct pathologist to pathologist referrals of difficult or equivocal cases) of PHM and CHM in relation to final review diagnosis.
Results: During the period 1988 to 2004, the annual number of histopathological cases reviewed (registrations and referrals) has increased from around 150 / yr to almost 1,000/ yr. Of 5,750 registered cases since 1992, 4,963 (86%) were PHM and 787 (14%) CHM. Of those with a registration diagnosis of CHM, 38 (4.8%) were reclassified as PHM and 12 (1.5%) as non-molar miscarriage. Of the 4,963 with a registration diagnosis of PHM, 1,062 (21.4%) were reclassified as early complete moles and 715 (14.4%) as non-molar miscarriage.
Conclusion: Central histopathological review is important for provision of accurate risk data and prognosis in pregnancies complicated by hydatidiform mole. The majority of cases with a local diagnosis of CHM represent true CHM, whereas more than one third of PHM registrations have the diagnosis modified on review. Recognition of the histopathological features of CHM evacuated in early pregnancy, as opposed to the later second trimester, appears to be an area of uncertainty for general pathologists which specialist centres should attempt to address.

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Diagnosis Of Intraplacental Choriocarcinoma And Relationship With Infantile Choriocarcinoma: Four Additional Cases

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Introduction: The development of persistent gestational trophoblastic disease following an apparently uncomplicated term pregnancy is well-recognized, however reports of confirmed intraplacental choriocarcinoma (IPCC) are rare.
Methods: A search of the histopathology database of a regional Trophoblastic Disease Unit was performed to identify cases of histologically reviewed intraplacental choriocarcinoma.
Results: During the search period (1986-2003 inclusive), 11,223 cases were reviewed, of which 4 (0.04%) IPCC were identified, all occurring in third trimester placentas, 2 associated with stillbirth and 2 with metastatic maternal disease. In all, macroscopic examination of the placenta was unremarkable, with small nondescript lesions being identified, initially thought to be fresh infarcts or intervillous thrombi, histological examination demonstrating the presence of focal IPCC.
Discussion: Review of the literature demonstrates primary IPCC may be associated with intrauterine death or fetal distress in 15% of cases, but the majority are initially asymptomatic, the diagnosis only being made following histopathological placental examination. Furthermore, metastatic maternal disease is present in 60% of cases at diagnosis. Primary neonatal choriocarcinoma (NCC), thought to be metastatic from the placenta in most cases, presents with anemia, failure to thrive and hepatomegaly, the liver being involved in 80%. Metastatic maternal CC is present in 60% of such infantile cases, however, the mother is usually clinically asymptomatic during pregnancy, maternal disease being detected following diagnosis of the neonate. In one case of NCC, a small intraplacental choriocarcinoma, identical to those described in our series, was identified.

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Subsequent histological diagnosis, after curettage or biopsy diagnosis of atypical hyperplasia of the endometrium

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Atypical hyperplasia (AH) of the endometrium is associated with endometrioid carcinoma (EC) A spectrum of atypia and suspicion for EC is seen in the diagnostic specimen. The management appropriate for these patients is unclear. In this centre, after multidisciplinary team review, patients with atypia of moderate/severe degree are offered hysterectomy. Those with mild atypia are treated similarly or offered alternative treatment e.g. intrauterine system containing progestogen.

Methods: AH cases over three years were reviewed. The original biopsy was compared with the hysterectomy specimen or subsequent biopsy.

Results: Of 23 cases, 20 underwent hysterectomy; eight (40%) had EC in the resection specimen. All were grade 1, and the majority low stage (three 1a, three 1b, one 1c, and one 2b) Twelve (60%) had AH alone in the resection specimen.

The pathology report and diagnostic specimen were reviewed. Of 8 cases with subsequent EC, all but one included the statement "suspicious for carcinoma" or "at least" AH, and review of all biopies confirmed moderate/severe dysplasia. However, of 12 cases with AH alone on resection, 5 included a similar statement.

Three patients were treated by progestogen coil. In subsequent biopsy one showed no evidence of hyperplasia, the other complex hyperplasia without atypia.

Conclusion: Many patients with AH have EC. The degree of suspicion for carcinoma in the biopsy does not correlate well with the subsequent findings; and the present treatment policy of hysterectomy for AH is appropriate.

In selected patients with mild atypia, coil treatment can reverse atypia and may be an alternative treatment to hysterectomy.

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Recurrent Psammoma Bodies In Cervical Cytology Smears: What Is Its Significance In A Postmenopausal Female?

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Aim: To describe the cause of psammoma bodies (PB) in a post-menopausal female.
Case Report: A 55-year-old post-menopausal female had PBs and atypical cells in a routine cervical smear in 2000. Subsequent smears over the next four years (n=4) continued to demonstrate the presence of PBs and atypical cells, the latter were thought to be glandular. She was thoroughly investigated clinically and radiologically with no evidence of an underlying malignancy. Cervical biopsies and endometrial curettage were non-contributory. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed in 2004, following extensive discussions with the patient.
This excision biopsies showed small bilateral ovarian serous cystadenofibromas with PBs present in the peritoneal washings. Histology demonstrated that the atypical cells were mesothelial cells that appeared to adhere to the surface of the PBs as they were extruded.
Conclusion: PBs are described in a wide range of both benign and malignant conditions, most frequently affecting the female genital tract. They often involve serous type epithelium. . Most studies published to date have found postmenopausal status to be a significant predictor of the likelihood of PBs being associated with malignant pathology. This case represents a possible pitfall in the assessment of the significance of PBs in a smear and the challenge to patient management and patient choice in this context.

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Antepartum Haemorrhage Due To Florid Cervical Decidual Reaction.

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Aim: We describe a patient presenting with antepartum haemorrhage and a colposcopic lesion clinically resembling invasive cervical cancer.
Case report: A 28 year old woman presented with unprovoked painless antepartum haemorrhage (APH) at 28 weeks of pregnancy. She had had a miscarriage in her first pregnancy. Clinically the APH was thought to be as a result of placenta praevia or abruptio placenta. Ultrasound revealed a live single fetus; the placenta was not low-lying or abnormal.
A warty lesion, with atypical vessels, that bled on contact was seen on speculum exam; colposcopy revealed a lesion resembling an invasive cervical cancer extending all around the cervix and occluding the canal.
Histological examination of a cervical biopsy showed a florid cervical decidual reaction with focal surface ulceration and prominent neovascularisation accounting for the cervical growth and haemorrhage.
Conclusion: We describe a rare an unusual cause of APH, due to cervicitis decidualis, suggesting abnormal placentation clinically and invasive cervical cancer colposcopically.

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Endometrial Metastasis Of Breast Cancer Is Rare

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Aim: to determine the frequency of metastatic breast cancer in endometrial biopsy material.
Materials and Methods: We reviewed the histology reports of all endometrial pipelle and curettage specimens which were SNOMED coded as adenocarcinoma (M81403) or metastatic carcinoma (M80106) between 1996 and 2004 (inclusive) to identify cases reported as metastatic breast carcinoma.
Results: We identified 350 cases of endometrial carcinoma in this period, of which only three cases represented metastatic breast carcinoma. These were seen in patients aged 80 (BMI 35; para 4), 64 (BMI 33; para 2) and 46 (BMI 29; para 1) years, 5 years, 12 years and 7 years following their primary breast carcinoma, having been on Tamoxifen for 5, 5 and 7 years respectively. The first patient presented with postmenopausal bleeding, the second with a pelvic mass and the third had a routine endometrial ultrasound scan for long-term tamoxifen therapy. The former two patients had grade 2 and grade 3 invasive duct carcinomas and the third a grade 2 lobular carcinoma. The metastatic tumours were ER-positive in all but the patient with the pelvic mass
Conclusion: Both breast and endometrial carcinomas are putative 'hormonal' cancers having certain risk factors in common including early menarche, late menopause, obesity, incessant ovulation, nulliparity and oestrogen ingestion. Despite these similarities metastatic breast cancer to the endometrial cavity is rare. However, a low threshold for formal gynaecological assessment is recommended for women who, following breast cancer, present with abnormal vaginal bleeding.

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An Audit of Negative LLETZ Biopsies

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LLETZ biopsy is the method of treatment for CIN. In 2003, 415 LLETZ biopsies were examined at Bristol Royal Infirmary performed for abnormal cervical smears. 84 LLETZ had no demonstrable CIN. We have audited and re-examined LLETZ biopsies of these 84 patients. In 31 patients LLETZ were performed for low grade smear abnormalities. 11 negative LLETZ were preceded by either positive punch or positive LLETZ biopsies. 34 patients had high grade dyskaryosis in smears but LLETZ were negative for dysplasia. There was squamous metaplasia in 27 cases (mature in 20 and immature in 7), koilocytosis in 9 cases, cervicitis in 6 cases and microglandular hyperplasia in 1 case.

Review of smears in these 34 patients demonstrated that original smear was overcalled for dyskaryosis in 6, 2 smears were reported as dyskaryosis but were technically inadequate. In 2 cases the original diagnosis were suspicious of high grade dyskaryosis but no definitive diagnosis was made. In 20 patients the reviewed smear matched the original smear diagnosis. Among these 20 patients, 45% (9/20) had koilocytosis, 30% (6/20) had immature squamous metaplasia, 30% (6/20) had cervicitis.

Our audit therefore suggests that koilocytosis, immature squamous metaplasia and cervicitis are the major causes of false positive smears in cervical pathology.

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Audit Of Turnaround Time For Reporting Of Cervical Biopsies And LLETZ Specimens

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The colposcopy and programme management guidelines hold best practice as 90% of patients undergoing colposcopy are being informed of their results within four weeks. The Gynaecologists were concerned that they were unable to meet this goal due to delays within the pathology department.

Therefore, a prospective audit was carried out for ten weeks. 153 specimens (49 LLETZ, and 105 cervical biopsies) were identified, to assess turnaround time, and the presence and location of delays. The time taken in the laboratory, awaiting reporting, with the secretarial staff, and awaiting authorisation was recorded by means of a form which travelled with the request card through its journey until filing.

Results: Only 39 cases (25%) were completed within one week, and 17 (11%) took longer than three weeks. The average turnaround time was 12.5 days. Processing in the laboratory took an average of 2.7 days with 24% taking 4 days or more. Average reporting time was 3.8 days with 15% taking longer than one week. Average time with the secretaries was 2.8 days, and average authorisation time 1.4 days.

Conclusion: Significant delays were identified within the system at a number of points, which will be addressed comprehensively.

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p16 Immunohistochemistry Distinguishes Endocervical From Endometrial Adenocarcinoma Invading Cervix

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Invasive adenocarcinoma within the endocervix may represent either primary endocervical adenocarcinoma or downward spread from primary endometrial adenocarcinoma. Given that these two cancers can sometimes appear morphologically very similar on H&E stains, the use of p16 immunohistochemistry (Novocastra clone 6H12) was evaluated as a potential diagnostic marker that may help in making this distinction. Similarly, cervical glandular intraepithelial neoplasia (CGIN) may be difficult to distinguish from tubo-endometrioid metaplasia (TEM) and p16 immunohistochemistry was also evaluated in this setting. All (11/11) cases of high grade CGIN showed strong, diffuse positivity for p16 in 100% CGIN cells. Whereas none (0/12) of the TEM lesions showed this strong diffuse pattern of p16 immunostaining, but they did demonstrate a patchy, moderate cytoplasmic p16 staining pattern. All (9/9) primary endocervical adenocarcinomas showed strong, diffuse p16 positivity in 100% cells, very similar to the pattern seen in high grade CGIN. In contrast, 9 out of 10 primary endometrial adenocarcinomas growing into the cervical stroma showed a different staining pattern for p16 that was patchy and weak to moderate in strength (with only very occasional strongly staining cells). The remaining 1 (out of 10) primary endometrial cancer showed a mixed pattern of partly patchy/moderate and partly strong/diffuse, but even this was distinguishable from primary endocervical adenocarcinoma. We conclude that p16 immunohistochemistry is a useful adjunct to aid distinction between endocervical and endometrial adenocarcinomas and to differentiate high grade CGIN from TEM.

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Immunohistochemical Expression of Estrogen Receptors (ER) and Progestrone Receptors (PgR) in Cervical Adenocarcinoma and its precursors Using Tissue Microarray (TMA) Technology

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Few studies have investigated the expression and clinical significance of (ER) and (PgR) in adenocarcinoma of the cervix. In this study we examined expression of ER and PgR in a series of cervical adenocarcinomas and precursors, using TMA technology. TMA blocks were constructed using paraffin-embedded, formalin-fixed tissues from 273 samples derived from 16 normal cervical biopsies, 119 cases of invasive adenocarcinoma and 20 high grade CGIN. Fresh 3-µm sections were cut and immunostained with ER and PgR antibodies, and expression was correlated with clinicopathological variables, including histological subtypes of adenocarcinoma. In 139 patients with invasive or preinvasive adenocarcinoma, ER expression was positive in 64 (47%) with 2 cases not available and PgR was positive in 50 (37%) with 4 cases not available. In all cases, normal endocervical cells and normal stromal cells of the cervix stained weakly for ER and PgR. The intensity and distribution of ER and PgR staining in the tumour tissue were more heterogeneous than that observed in the normal tissues. ER positivity was more frequent in invasive (50%) and early invasive (56%) cervical adenocarcinomas than in AIS (28%) which suggests that high ER levels are possibly associated with more advanced lesions. Moreover, high ER and PgR levels were more frequent in minimal deviation adenocarcinoma and adenosquamous carcinomas, consistent with the suggestion that ER and PgR expression are more frequent in tumours of worse prognosis. Our findings suggest that ER and PgR status may be useful markers in women with cervical adenocarcinoma. Further assessment of the impact of ER and PgR status on the clinical outcome of patients with primary invasive adenocarcinoma of the cervix in larger case series is needed.

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Ligneous cervicitis; is it the emperor's new clothes? Case report and analysis of possible aetiology

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Ligneous disease is a rare form of idiopathic recurrent, chronic woody pseudo-membranous inflammation that usually affects the conjunctiva; it is refractory to all forms of therapy. The pseudo-membrane is composed of amorphous eosinophilic amyloid-like material.
Plasminogen deficiency is the most acceptable aetiological factors amongst authors in this field. However, the underlying pathogenesis is still unclear neither has an effective method of treatment emerged. Surgical removal, cyclosporine, anti-inflammatory agents and antibiotics have been tried with limited or no success.
Extra-conjunctival lesions have been reported affecting mainly the upper respiratory tract, gingiva and the female genital tract particularly the cervix and vagina. In the few cases reported of female genital tract involvement, all had either previous or subsequent ocular involvement.

We would like to report this unusual case of ligneous disease localised to the female genital tract for the last 20 years with repeated presentation as post coital bleeding albeit without any ocular or upper respiratory tract involvement. We also offer analysis of different possible aetiological factors with a prospect for wider field of research in this area.

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Comparison of Examination Of The Entire Cervix With Routine Cervical Sampling in Hysterectomy Specimens From Women With Endometrial Carcinoma

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Assessment of cervical involvement by endometrial carcinoma is important for accurate tumour staging with consequent management implications. In routine practice in our hospitals, examination of the cervix in hysterectomy specimens from women with endometrial carcinoma is confined to midline blocks from anterior and posterior lips.

The aim of this study was to investigate whether examination of the entire cervix in hysterectomy specimens from women with endometrial carcinoma leads to increased detection of cervical involvement compared with routine sampling of midline blocks.

Hysterectomy specimens for endometrial carcinoma received over a 2-month period were included. Routine midline blocks were sampled and the entire cervix was then embedded in parallel slices. Cervical involvement was assessed by 2 individuals initially in routine midline blocks, and compared with involvement in blocks of the entire cervix.

Our preliminary results on 12 hysterectomy specimens show cervical involvement in 3 cases, which was identified in the routine 2 midline blocks. The extra blocks provided no further information in either the 3 positive or 9 remaining cases with no cervical involvement.

These results support continuing the practice of examining routine midline blocks rather than sampling of the entire cervix in hysterectomy specimens from women with endometrial carcinoma.

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Assessment Of Recurrence Following Cervical Loop Excision In Relation To Involvement Of Different Resection Margins

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Current guidelines for reporting cervical large loop excision of transformation zone (LLETZ) require assessment of ectocervical, endocervical and deep lateral margins, which may involve further technical procedures, such as turning of lateral blocks or additional levels. Our aim was to assess recurrence of dysplasia following LLETZ.

All LLETZ received over 6 months were included. Involvement of ectocervical, endocervical and deep lateral margins was recorded as was follow-up cytology and biopsy reports for 3 years. Rates of recurrent disease were compared after complete and incomplete excision of dysplasia.

Of 315 LLETZ, excision was complete in 124 (39%), incomplete in 174 (55%) and not assessable in 17 (5%). Excision was incomplete at the ectocervical margin in 67.8%, endocervical margin in 14.4% and at >one margin in 18%. Of these follow-up was normal in 73.7%, 68% and 74.2%, respectively. No case showed involvement of deep lateral margin alone. Follow-up over 3 years for dysplasia-dyskaryosis/ any abnormality (including borderline smear or ECUS) was: complete excision 5.6% / 8.9%, involved ectocervical margin 17.8% / 26.3%, endocervical margin 8% / 32%, >one margin 6.5% / 25.8%), non-assessable 35% / 100%. Only nine cases required further LLETZ.

Recurrence of dysplasia-dyskaryosis after incomplete LLETZ is low, regardless of which margin is involved.

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$p57^{KIP2}$ Expression Is A Useful Adjunct For Differentiating Early Complete And Partial Hydatidiform Moles

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Differentiation of hydatidiform mole from hydropic abortion is difficult. The distinction of early complete from partial mole is particularly problematic. $p57^{KIP2}$, a cyclin-dependent kinase inhibitor, is paternally imprinted (maternally expressed). Recent studies have shown expression of $p57^{KIP2}$ in chorionic villi of spontaneous abortions and partial but not complete moles.

40 gestational products (11 complete moles, 15 partial moles, 14 non-molar on consensus diagnosis) reviewed by the local gynaecological pathology consensus group over 18 months were immunostained for $p57^{KIP2}$. Villous stroma and cytotrophoblast were examined for $p57^{KIP2}$ expression.

$p57^{KIP2}$ staining was positive in all cases originally diagnosed as non-molar or partial mole. 2/11 cases originally designated as complete mole were $p57^{KIP2}$ positive; on review one was reclassified as a partial mole, while histological diagnosis of complete mole in the other was upheld. Some complete moles showed scanty reactivity with villous cytotrophoblast in rare villi. One hydropic abortion showed very weak $p57^{KIP2}$ expression, due to attenuation and loss of villous cytotrophoblast.

Consensus group reporting of problematic products of conception is successful. $p57^{KIP2}$ staining did not detect any additional complete moles. $p57^{KIP2}$ expression is useful to aid differentiation of early complete from partial hydatidiform moles but expression may be weak in hydropic abortions.

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Association of Clinical Electron Microscopists
Companion Meeting: Thursday 7 July 2005

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Borderline Smears and High-Grade Histology – Are We Getting It Right?

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The borderline category for cervical smears is used for cases where the presence of dyskaryosis is uncertain. Smears with borderline changes are not referred for colposcopy immediately, and where the histological changes are high-grade, treatment is delayed.

This study aimed to review cases with borderline cytology and high-grade histology, determine factors leading to a borderline report and find indicators of high-grade histology in smears.

27 such cases were found in a six-month period. These were seeded among 54 random smears and reviewed by two consultants and four cytoscreeners. Factors possibly contributing to a borderline report were also reported.

In 9 of the cases, a majority of the reviewers agreed that the changes were borderline. In the remaining cases, the majority opinion was high-grade changes in 4 cases, mild dyskaryosis in 1 case and no consensus in 13 cases. However, in 10 of these 13 cases, a majority agreed there were low-grade (borderline or mild) changes. The cases consistently being reported as borderline had less than 20 abnormal cells on the smear. Obscuration of cells also contributed to a borderline diagnosis.

In conclusion, certain cases with high-grade histological changes will be reported as borderline change, usually due to an insufficient number of abnormal cells on the smear and sometimes due to obscuration. This should be taken into account when managing borderline nuclear change.

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An Audit of Endometrial Cancers in 5290 Unselected Curettings

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Our aim was to ascertain the distribution of endometrial neoplasms together with their subtypes, stage and grade in a large teaching hospital.

The reports of 5290 unselected endometrial curettings received in our department between 1996 and 2002 were reviewed. 151 curettings showed malignancies and 43 showed atypical hyperplasia.

The reports of the 145 subsequent hysterectomies of these 194 abnormal curettings were reviewed. 119 of the hysterectomies showed malignancies. These included 8 carcinomas seen in 29 hysterectomies done for atypical hyperplasia on curettings. Overall, endometrioid carcinoma was the most common type of malignancy seen (87). The other malignancies seen were papillary serous (7), carcinosarcoma (6), clear cell carcinoma (3), metastatic carcinoma (3) and miscellaneous malignancies (13). The grade and stage of the endometrioid cancers is shown in the following table:

	Stage 1	Stage 2 or higher
Grade 1	35	11
Grade 2	18	9
Grade 3	7	6

Vascular invasion was seen in 19.6% of grade 1 cancers in comparison with 53.4% of grade 3 cancers.

The vast majority of the cancers were found to be grade 1 endometrioid adenocarcinomas. It was of interest to note the high percentage of grade 1 cancers showing vascular invasion.

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Prevalence and Significance of Metaplasias in 5290 Endometrial Curettings in a Large Teaching Hospital

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Metaplasias are seen not infrequently in the endometrium. We aimed to determine the prevalence and significance of metaplasias in a non-selected series of 5290 curettings in a 7-year period.

The histology reports of 5290 curettings submitted between 1996 and 2002 were reviewed to detect the presence and type of metaplasia with any associated pathology.

238 curettings (4.5%) showed the presence of one or more types of epithelial metaplasia. 38 (15.9%) of these were associated with hyperplasias and 35 (14.7%) with cancers. Squamous metaplasia was the most common type of metaplasia associated with atypical hyperplasia and cancer. The prevalence of squamous metaplasia was highest with atypical hyperplasia (6 cases) compared to simple (0 cases) and complex (5 cases) hyperplasia. 13 (43.3%) of the grade 1 endometrioid adenocarcinomas were associated with squamous metaplasia in comparison to only 5 (16.7%) of grade 3 carcinomas.

Our study supported the findings of previous studies in the recognition of the association of squamous metaplasia with hyperplasia. We found an increased association of squamous metaplasia with increasing complexity of endometrial hyperplasia and endometrioid adenocarcinoma. Squamous metaplasia may therefore be a potentially useful marker of neoplasia

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Cystic Adenomatoid Tumour of the Uterus

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Adenomatoid tumour of the uterus is a relatively rare neoplasm which is confined to the genital tract of both genders. The tumour is often discovered as an incidental finding and is mesothelial in origin, characterised by benign behaviour.

The morphological appearances include small nodules, fibroid-like masses, diffuse myometrial involvement and cystic spaces resembling a lymphangioma.

We report a case of adenomatoid tumour of the uterus composed entirely of macrocysts lined by bland epithelium. Immunohistochemically these cells were positive for calretinin, CK5/6 and cytokeratin (MNF116). CD31 and CD34 were negative.

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Implementation and Evaluation of a new Automated Interactive Image Analysis System for Cervical Cytology.

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The ThinPrep® Imaging system consists of an Image Processor and one, or more, Review Scopes (RS). The system makes use of computer imaging to select fields of view for presentation to a cytotechnologist on a RS. Slides used with this system must first be prepared on a ThinPrep® 2000 or 3000 processor and stained with a new ThinPrep® Pap stain. In clinical trials it was shown to have diagnostic equivalence with manual screening.

In this study we compared the RS screening result from the 22 fields of view (FOV) only, as presented by the Autolocate mode of the RS, with independent primary screening of 6000 ThinPrep slides. Sensitivity, specificity and positive predictive value (PPV) were calculated for each method. Differences in both methods were analysed for statistical significance using a chi-square test. A p-value of less than 0.05 was considered statistically significant.

Results showed that the diagnostic sensitivity of the Imager was 85%, diagnostic specificity was 97.6% and PPV was 84.9%. The diagnostic sensitivity of primary screening was 87.3%, diagnostic specificity 98% and PPV 87.3%. There was no statistical difference between both sets of results ($p > 0.05$).

These results show that the Imager performs at a level comparable to that of manual screening. We believe that the Imager will make a valuable contribution to any cervical screening programme and will greatly improve productivity.

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Is There A Benefit From Taking Histology From Unfixed Hysterectomy Specimens?

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Background: The conventional method for taking blocks from hysterectomy specimens for endometrial carcinoma requires adequate fixation before block selection. This results in variable fixation of the tumour.

Method: A retrospective audit was performed comparing two laboratories within the same NHS trust, one where blocks are taken from fresh tissue (51 cases) and one where tissue is fixed before trimming (49 cases). Each case was scored as 1 = poor preservation, 2 = some poorly preserved areas or 3 = well preserved throughout.

Results: 71% of cases where the blocks were taken from fixed tissue showed variable preservation compared with unfixed cases where only 12% showed focally poor preservation. However none of the cases reviewed were ungradable.

Conclusion: Our results suggest that taking blocks from fresh tissue yields better preservation of endometrial morphology. This is essential for FIGO grading as this requires adequate preservation of nuclear morphology. This technique also supports a rapid specimen turn-around time.

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MCM3 ASSESSMENT IN CERVICAL NEOPLASIA

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Background: Minichromosome maintenance (MCM) proteins play essential roles in eukaryotic DNA replication. Several studies have highlighted the potential of these proteins as molecular markers of dysplastic and malignant cells in histopathological diagnosis. The mode of expression of MCM3 and its clinical significance in normal, dysplastic and malignant cervical lesions remains to be elucidated.

Design: Immunohistochemical analysis with MCM3 (mouse monoclonal antibody -Dakocytomation) was examined in formalin fixed paraffin embedded tissue from the following (n=50): normal cervix, CIN1 CIN2, CIN3, cGIN, invasive squamous cell carcinoma and adenocarcinoma (including villoglandular adenocarcinoma) of the cervix.

Results: Normal cervix showed MCM3 positivity in the nuclei of the basal proliferative layer with occasional staining of normal endocervical glandular cells. Nuclear staining of MCM3 protein was observed in all grades of squamous and glandular neoplastic lesions. Strong nuclear MCM3 staining was evident in invasive cervical squamous cell carcinomas and adenocarcinomas, and correlated with the degree of tumour differentiation. Poorly differentiated squamous and glandular invasive tumours typically demonstrated higher MCM3 staining intensity.

Conclusion: The results of this study indicate the potential use of MCM3 as a molecular biomarker for diagnosis of squamous and glandular cervical pre-neoplastic or neoplastic lesions.

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A novel combination of biomarkers for cervical glandular neoplasia

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Background: Accurate diagnosis of cervical glandular premalignant and malignant lesions is fraught with difficulty. To aid the distinction, our group has evaluated the use of $p16^{INK4A}$, mini chromosome maintenance proteins (MCM) 3 and 5, cell division cycle protein 6 (CDC6), MIB-1 - marker of cell proliferation, and bcl2- a proto-oncogene as a panel of cervical neoplastic biomarkers.

Design: 60 cases of cervical glandular lesions including normal, cGIN, invasive adenocarcinoma, benign glandular lesions (microglandular hyperplasia, reactive atypia, TEM) were assessed immunohistochemically with antibodies to $p16^{INK4A}$, MIB1, bcl2, MCM3, MCM5 and CDC6. A score of 0-3 was given for each antibody in each case.

Results: Immunopositivity was noted in the majority of premalignant and malignant cervical lesions for $p16^{INK4A}$, MIB1, MCM 3, MCM 5 and CDC6 while bcl2 was negative or focally positive. Benign lesions were strongly positive for bcl2 with focal positivity for $p16^{INK4A}$. MIB1 was negative in benign and normal cervical glands while MCM 5 and CDC6 showed variable staining.

Conclusion: The diagnosis of cervical glandular dysplasia gives rise to multiple dilemmas both in the terminology of classification and its accurate diagnosis. We propose that panel of biomarkers for the diagnosis of difficult cases.

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Case Report: An Unusual Combination Of Endometriotic Polyp In The Caecum And Malignant Mixed Mullerian Tumour Arising In An Ovarian Endometriotic Cyst

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Malignant transformation of endometriosis has been reported, as has polypoid endometriosis in the gastrointestinal tract. This case demonstrates two synchronous, uncommon manifestations of endometriosis in a woman aged 69 years with a longstanding exogenous oestrogen source.

The patient underwent hysterectomy and right salpingo-oophorectomy for endometriosis 30 years prior to detection of a left ovarian cystic mass. At laparotomy a polyp was noted on the serosal surface of the caecum, which was considered to be neoplastic and right hemicolectomy and left salpingo-oophorectomy were performed. Histology of the ovarian mass revealed an endometriotic cyst. Within the cavity of the cyst was a malignant mixed mullerian tumour (MMMT) without heterologous elements showing malignant stromal elements and epithelial elements ranging from unremarkable proliferative endometrium through severe cytological atypia to endometrioid-type adenocarcinoma. The caecal serosal polyp was related to several mucosal polyps and showed widespread endometriosis extending from the serosa to the mucosal surface.

Patients receiving prolonged oestrogen therapy are at risk of endometriosis at various sites including ovary and large bowel; the latter may form polyps, which can be misinterpreted clinically as cancer. Although ovarian endometriosis is typically associated with clear cell and endometrioid carcinoma, MMMT is a recognised rare complication.

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Lobular Carcinoma In Situ: Expression of Chromosome 16q Genes CDH1, DPEP1, DERPC and CTCF

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Lobular carcinoma *in situ* (LCIS) comprises 5.7% of breast malignancies and is a risk factor for development of invasive carcinoma. Loss of chromosomal material at 16q occurs in LCIS and is an early genetic event in invasive breast cancer development. E-cadherin (CDH1), CCCTC-binding factor (CTCF), Decreased Expression in Renal and Prostate Cancer (DERPC) and Dipeptidase 1 (DPEP1) are all located within commonly lost areas of chromosome 16 and are potential tumour suppressor genes.
Immunohistochemistry for CTCF and E-cadherin was performed on LCIS (n=55) and normal breast (n=6) cases. All normal cases showed strong E-cadherin expression in the lobules, whereas LCIS showed significantly reduced or negative expression. A similar pattern of expression was seen with CTCF.
Cells from LCIS cases (n=11) and normal lobules (n=6) were microdissected and expression of target genes were quantified using real-time PCR. CDH1, CTCF and DPEP1 expression was significantly lower in LCIS cases compared to normal cases. However, there was no difference in expression of DERPC between LCIS and normal lobules.
Loss of CDH1, CTCF and DPEP1 expression suggest they are potential tumour suppressor genes in breast cancer and may be potentially utilised as markers of predisposition of women diagnosed with LCIS to develop invasive carcinoma.

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Abstract Withdrawn

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Correlation Of Core Biopsy And Definitive Tumour Resection In Assessing Key Prognostic Parameters In Invasive Breast Carcinoma

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Core biopsy is a method of choice in the triple assessment of breast disease and is widely used in planning management of breast cancer. Our study looked at the reliability of core biopsy in predicting the grade and other prognostic parameters in the resected tumour.

250 subjects with core biopsy diagnosis of invasive breast cancer and with subsequent surgical resection were included. Comparison was made between the grade assigned to the core and to the resection specimen, for overall grade (1-3) and the components where documented. Statistical analysis was done using the Chi-square test.

Core biopsy and resection showed 77.1% ($p<0.001$) concordance for overall grade, with scores for grades 1, 2 and 3 being 83%, 73.1% and 72.7% respectively. The grade was underestimated in the core in 41 cases (16.7%), which was by a factor of one in 40 cases and by two in one case.
15 cases (6.1%) showed an overestimation of grade in core, all differing by a factor of one.

The core/resection concordance for tumour classification, in-situ carcinoma and lymphovascular space invasion was 88%, 55% and 70% respectively.

Conclusion: Our results show that pre-operative core biopsy is a reliable predictor of histological grade of invasive breast cancer.

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A Rare Case Of Fibromatosis In A Male Breast

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Aim: To describe a rare cause of a breast lump in an adult male.
Case Report: A 44 year old male presented with a three month history of a tender lump in the right breast which was clinically (P5) and radiologically (R5, U5) suspicious.
Fine needle aspiration cytology showed a dispersed population of mixed bipolar and unipolar spindle cells with significant amounts of cytoplasm and little nuclear atypia. Collagenous stromal fragments were also present. This was reported as C4.
The subsequent excision specimen showed a firm 21mm diameter, irregular, spiculate white nodule which, histologically, was composed of short bundles of bland spindle cells within dense, focally keloidal, collagen bands. It had an irregular, "stellate" margin with chicken-wire type extension around adipocytes in the immediate vicinity of the lesional margins. Very occasional mitotic figures were present. The tumour was strongly vimentin-positive, focally SMA-positive, but was CD34-, CD31-, desmin-, S100-, oestrogen-, progesterone-, cytokeratin- and factor XIIIa-negative.
He had a calcaneal nodule removed from the left foot in 1996, with the histological features of fibrous dysplasia.
Conclusion: Fibromatosis of the male breast is a rare neoplastic lesion with a tendency for local recurrence. It mimics breast carcinoma both clinically and radiologically. The cytology is non-diagnostic with definitive diagnosis and treatment relying on complete excision and histological evaluation

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Should B3 and B4 Breast Needle Core Biopsies Be Double Reported?

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The triple approach in diagnosis of breast lesions is well established and performance targets are specified by the NHSBSP for breast needle core biopsies. Data was collated from the histopathological reports of all symptomatic and screening patients undergoing needle core biopsy from April 1st 2002 – March 31st 2003 (n=177). The performance figures were subsequently calculated. All of the B3/B4 diagnoses (n=12) were subject to slide review by two pathologists. The department achieved all of the recommended thresholds except for full specificity which was 69.3% (recommended minimum threshold >75%). Review of cores from the B3/B4 cases revealed both tissue sampling problems and that additional levels might have assisted in diagnosis. Scanty tissue cores were the main cause of inaccurate diagnosis. On review, 2/12 (17%) of cases were upgraded from B3 to B5a and one case downgraded from B4 to B3.

Conclusion: Improvements in performance figures could potentially be achieved by performing at least six further tissue levels and by double reporting B3/B4 cases *as well as* MDT case review. As the overall number of these cases is small (less than 10%) this proposal for double reporting of B3/B4 cases is unlikely to have significant resource implications.

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Correlation Of Hormone Receptor Status In Breast Cancer Between Core Biopsy And Surgical Resection

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An audit of the correlation between oestrogen receptor (ER) and progesterone receptor (PR) status on core biopsy and subsequent surgical resection was performed in 120 consecutive breast cancer cases at Bradford Teaching Hospitals NHS Trust. Scoring was performed according to the Allred method in which each sample is given a score between 0 and 8, correlating with a likely response rate to endocrine therapy in advanced breast cancer of 0% (negative, score =0), 20% (weakly positive, score = 1-3), 50% (moderate, score 4-6) and 75% (strongly positive, score = 7 or 8) respectively. Cases where a lack of concordance in predictive grouping was identified between core and excision specimen were reviewed and repeat immunohistochemistry performed where appropriate.

In 108 (90%) of cases for ER, and 102 (85%) for PR there was no difference in predictive grouping between core biopsy and surgical resection. Two cases (1.67%) were complete false negatives and 2 cases were complete false positives for ER. Three cases (2.5%) were false negative and 3 false positive cases for PR. The reasons for the lack of concordance were established.

Our results indicate that moderate or strong ER positivity on core biopsy may be sufficiently robust to determine the need for endocrine therapy. Repeat immunohistochemistry should be performed on the surgical specimen if the core biopsy is negative or weakly positive.

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A Case of Fibroepithelial Stromal Polyp Presenting as Nipple Mass

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Introduction: Fibroepithelial stromal polyps (FESPs) are benign lesions that represent an entity in the group of vaulvovaginal mesenchymal lesions. They often pose diagnostic problems for the practising pathologist since they exhibit a wide range of histological appearances. These lesions, apart from being uncommon, have overlapping morphological features with other mesenchymal lesions including angiomyofibroblastomas, aggressive angiomyxomas, cellular angiofibromas, leiomyomas, superficial angiomyxoma, perineuriomas and neurofibromas. FESPs have not been described in the breast.

Case: This 45-year-old lady presented with a painless slowly growing nipple mass. Grossly, this was a polypoid mass that was attached to the nipple by a long pedicle. The cut section was firm and glistening. Microscopically, the lesion was fairly circumscribed but with no clear demarcation from the overlying epidermis. The pedicle contained unremarkable fibromascular tissue of the nipple. The lesion was variably cellular comprising a haphazard proliferation of bland spindle and stellate cells. Occasional multinucleate stromal giant cells were noted. Immunohistochemistry showed lesional cells to be focally immunoreactive for SMA and CD34 but negative for desmin, caldesmon, EMA, S100, ER, PR and factor XIIIa. The histology and immunohistochemistry was reviewed and the diagnosis of a fibroepithelial stromal polyp of similar histological features to those of the genital area was made.

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Aneusomy X in breast cancer

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Analysis of X chromosome inactivation has been used extensively as a marker of clonality in studies of neoplasia, but can be difficult to interpret. Contamination of homogenised tissues by non-tumour cells may obscure monoclonality and inadequate data about patch size in most normal tissues compromises data interpretation. *In situ* assays of X inactivation and X chromosome parental origin would address these issues but the latter does not yet exist. As a preliminary step we decided to investigate X chromosome dosage and inactivation status in breast cancer by *in situ* methods.

X chromosome copy number was visualised by fluorescence *in situ* hybridisation and we sought to visualise XIST RNA coating the inactive X chromosome by *in situ* hybridisation. Ten samples of normal breast tissue and 50 breast cancers were analysed in paraffin sections.

The mean X chromosome copy number visualised (2.1 ± 0.47 per cell) was significantly higher in breast cancers than normal breast tissue (1.55 ± 0.55; $P < 0.001$) and markedly skewed towards higher copy numbers (median 1.95, interquartile range 1.85-2.25). There was considerable heterogeneity within individual tumours. Reliably visualisation of XIST RNA coating the inactive X chromosome was not achieved.

Aneusomy X in breast cancer is another potential confounder of conventional clonality assays and suggests a role for X-linked genes in breast carcinogenesis (interactions between XIST RNA and BRCA1 have been described). A reliable *in situ* assay of X inactivation status ought to be achievable. *In situ* assay of parental X chromosome origin remains a more distant goal.

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Epigenetic modification of Heparan Sulphate D-Glucosaminyl 3-O-Sulphotransferase (HS3ST) genes in breast cancer

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Methylation of promoter CpG islands in human cancers is associated with histone modification and down-regulation of gene expression. Using methylation-specific PCR (MS-PCR) we have shown frequent promoter methylation in members of the heparan sulphate D-glucosaminyl 3-O-sulphotransferase (*HS3ST*) gene family. HS3ST enzymes in the Golgi apparatus add 3-O sulphate groups to glucosamines in heparan sulphate proteoglycans (HSPGs), which are found on the cell surface and in the extracellular matrix. Enzymes of this family are substrate-specific and the modifications they catalyse determine affinity of HSPGs for growth factors, cell-surface receptors and other molecules. Promoter methylation in breast cancer was most frequent in *HS3ST2* (40/69), *HS3ST3B* (28/69) and *HS3ST4* (32/69).
Functionally significant promoter methylation is generally associated with dense CpG island methylation. Dense methylation was confirmed in every case examined by MS-PCR sequencing of *HS3ST2* and *HS3ST4* (10 tumours each). We have also explored the effect of promoter methylation on *HS3ST* gene expression in cell lines. Ten cell lines were screened for *HS3ST2* and *HS3ST4* promoter methylation. The ovarian cancer cell line A2780 has *HS3ST2* methylation and MCF7 cells have *HS3ST4* promoter methylation. In these cell lines RT-PCR showed that both unmethylated *HS3ST* genes were expressed but neither of the methylated *HS3ST* genes. Reversal of methylation by culture in 5-deoxyazacytidine restored *HS3ST* gene expression in both cell lines. These data strengthen the hypothesis that epigenetic modification of *HS3ST* genes is significant in the genesis of breast cancer, possibly through epithelial/stromal interactions.

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Pre-operative Diagnosis of R4 Screen Detected Breast Lesions: FNAC Versus WBNB – Comparison Of Performance Over 15 Years

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Screen detected R4 (radiologically suspicious, probably malignant) lesions are often small and difficult to sample. One advantage of Fine Needle Aspiration Cytology (FNAC) over Wide Bore Needle Biopsy (WBNB) is the ability to sample more widely.
The aim of this study was to compare the performance of these two methods in pre-operative diagnosis and to compare the performance of pre-operative diagnosis in R4 lesions with performance overall.
Over 15 years 2053 screen-detected R4 lesions were sampled yielding 1010 WBNB and 1239 FNAC. Performance parameters were calculated using the NHS BSP guidelines.
For R4 lesions, WBNB showed higher sensitivity (84% vs 57%) and specificity (56% vs 33%) and lower inadequate (7.8% vs 17.8%) and suspicious rates (5.9% vs 24.4%) than FNAC. Comparing performance parameters for R4 against all lesions, sensitivity and specificity were comparable, inadequate rates were lower for R4 lesions and suspicious rates were similar.
The results show that pre-operative performance parameters for R4 lesions are comparable to those obtained overall and that the superiority of WBNB over FNAC is maintained in the analysis of R4 lesions.

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Radial Scar Lesions Of The Breast: Analysis Of Cases Containing Occult Malignancy

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Radial scars (RS<10mm) and complex sclerosing lesions (CSL>10mm) present as impalpable, spiculated radio dense lesions on screening mammography radiologically indistinguishable from carcinoma (R4). If biopsy is not diagnostic of carcinoma (C5 or B5) the lesions are excised by wire guided localisation biopsy as up to 20% may contain occult malignancy.
This study reviewed cases of RS/CSL lesions with benign wide bore needle biopsy (WBNB) which contained malignancy on excision.
11 cases where malignancy was found on excision after WBNB (6 cases: B1, 2 cases : B2 and 3 cases : B3) were reviewed in detail. In 6 of 11 cases a radial scar or stromal sclerosis was seen in WBNB. Localisation biopsy showed DCIS in 6, DCIS with invasive carcinoma in 3 and invasive carcinoma in 2. In 9 of 11 cases needle tracks were identified missing the malignant epithelium by a mean of 5mm (median:4mm; range:1-20mm).
The study shows that RS/CSL may contain malignancy on excision after benign WBNB. In 7 of 9 cases the malignancy was missed by <6mm suggesting that multiple cores or mammotome would reduce the false negative rate. The use of WBNB instead of FNAC has not eliminated the problem of false negative biopsy in RS/CSL and excision is recommended.

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Glucose-Regulated Protein 78 (Grp78) is Highly Expressed in the Majority of Human Breast Cancers

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Grp78 is a molecular chaperone that binds to malfolded proteins and unassembled complexes. It is upregulated under conditions of stress including glucose deprivation. *In vitro* studies have demonstrated a correlation between increased expression of Grp78 and resistance to apoptosis by topoisomerase 2 directed drugs. There is only one published study investigating the expression of Grp78 in breast cancer and that only contained 14 tumours. In this study 395 invasive breast cancers (21% grade 1, 48% grade 2, 29% grade 3; 75% ductal NST, 10% lobular, 5% tubular, 10% other histological types) were sampled in quadruplicate in tissue microarrays, and immunohistochemistry for Grp78 was performed. The staining was scored semi-quantitatively with a cumulative total for 3 valid tissue cores from each case. There was strong immunohistochemical expression of Grp78 in 76% of tumours. There was no significant statistical relationship between expression of Grp78 and tumour grade (p=0.600), tumour size (p=0.492), lymph node metastases (p=0.179), vascular invasion (p=0.789), histological type (p=0.726), oestrogen receptor expression (p=0.074) or the Nottingham Prognostic Index (p=0.400). There was no significant relationship with survival in Cox regression analysis with NPI as the sole covariate (p=0.480). However given the high level of expression of Grp78 in the study population significant association with tumour subtypes is not expected. This study shows that expression of Grp78 is increased in the majority of breast cancers. This expression may lead to a resistance to apoptosis-inducing drugs but its cell surface expression may provide a therapeutic target.

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There is a General Loss of Expression of Receptor Activator of nFkB Ligand (RANKL) in Human Breast Cancers But Retention of Expression Has a Significant Positive Association With Expression of Oestrogen Receptor (ER)

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RANKL is essential for the development of lactating breast lobules in the mouse, RANKL knockout mice are unable to successfully nurse their young due to the lack of formation of functional breast lobules. We have shown that RANKL is expressed at the luminal surface of epithelial cells in normal adult breast tissue. RANKL mRNA is reported to be expressed in human breast tumours, but the relationship between this protein and breast cancer has not been investigated. 395 invasive breast cancers (21% grade 1, 48% grade 2, 29% grade 3; 75% ductal NST, 10% lobular, 5% tubular, 10% other histological types) were sampled in quadruplicate in tissue microarrays, and immunohistochemistry for RANKL was performed. The staining was scored semi-quantitatively with a cumulative total for 3 valid tissue cores from each case. There was strong immunohistochemical expression of RANKL in 14% of tumours with a strong positive association between RANKL expression and ER expression (p=0.036). There was no significant statistical relationship between expression of RANKL and tumour grade (p=0.050), tumour size (p=0.808), lymph node metastases (p=0.934), vascular invasion (p=0.062), histological type (p=0.367) or the Nottingham Prognostic Index (p=0.263). There was no significant relationship with survival in Cox regression analysis with NPI as the sole covariate (p=0.801). This study shows that expression of RANKL is lost in the majority of breast cancers and those that retain expression are likely to also express ER. Further studies are needed to investigate what role this loss of expression plays in tumour formation and progression.

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Tumour Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL) is Expressed in Human Breast Cancers And Has a Significant Positive Association With Tumours With Poor Prognosis

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TRAIL binds to cell surface receptors which activate caspases, leading to apoptosis. TRAIL is produced by intratumoural macrophages and may be the principal mediator of tumour cell death by these cells. However we have shown that TRAIL is also expressed in the myoepithelial cells of normal breast tissue. This study investigates the expression of TRAIL in breast cancer. 395 invasive breast cancers (21% grade 1, 48% grade 2, 29% grade 3; 75% ductal NST, 10% lobular, 5% tubular, 10% other histological types) were sampled in quadruplicate in tissue microarrays, and immunohistochemistry for TRAIL was performed. The staining was scored semi-quantitatively with a cumulative total for 3 valid tissue cores from each case. There was strong immunohistochemical expression of TRAIL in 30% of tumours. There was no significant statistical relationship between expression of TRAIL and tumour grade (p=0.159), tumour size (p=0.147), lymph node metastases (p=0.088), vascular invasion (p=0.567), histological type (p=0.770), and ER status (p=0.154) but there was a significant positive association with the Nottingham Prognostic Index (p=0.021) suggesting a multivariate relationship with grade, size and lymph node status. There was no significant relationship with survival in Cox regression analysis with NPI as the sole covariate (p=0.635). Although there were no significant associations with single variables the positive association with the NPI suggests that TRAIL expression is associated with tumours with a worse prognosis. TRAIL does not appear to be a useful prognostic factor or therapeutic target in breast cancer on its own but it may have utility in systems assessing the expression of multiple proteins.

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An Integrated Analysis of the Expression of Osteoprotegerin (OPG), Tumour Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL), Receptor Activator of nFkB Ligand (RANKL) and Oestrogen Receptor (ER) in Human Breast Cancer

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OPG binds to RANKL in bone where it regulates turnover. It is also a decoy receptor for TRAIL which inhibits TRAIL's apoptosis-inducing function on cancer cells so production of OPG might be a tumour cell survival factor. We are investigating whether these related proteins play any role in tumour genesis, progression or survival in the breast. We have shown that each protein is localised to a different cellular compartment in the breast – RANKL at the luminal surface of epithelial cells in lobules, TRAIL in myoepithelial cells and OPG only in epithelial cells with columnar change. We have performed immunohistochemistry for all 3 proteins and ER on 395 breast cancers in tissue microarrays, the relationship between each individual protein and clincopathological factors has been reported separately but an integrated analysis is now possible. TRAIL & RANKL showed significant co-expression (p<0.005, Jonckheere-Terpstra test) but there was no significant co-expression of OPG & TRAIL (p=0.511) or OPG & RANKL (p=0.347). There was significant co-expression of ER & OPG (p=0.007) and ER & RANKL (p=0.036) but not ER & TRAIL (p=0.154). Cluster analysis and visualisation of the subsequent hierarchical tree structure (Cluster & Treeview, EisenLab, University of California, US; de Hoon, University of Tokyo) for OPG, TRAIL and RANKL without ER showed a clustering of ER+ low grade tumours at one end of the spectrum and ER- high grade tumours at the other end but the discrimination was insufficient for clinical prognostication.

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The Role Of S100A9 In The Tumour-Suppressor Effect Of Normal Breast Myoepithelial Cells

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We previously have demonstrated that primary myoepithelial cells (MEC) derived from normal breast exert a broad tumour-suppressor effect, reducing breast cancer cell proliferation and invasion and enhancing apoptosis. To address the mechanisms involved, we performed cDNA microarray analysis on T47-D tumour cells co-cultured with MEC and compared the expression pattern to that obtained from T47-D cells grown alone. A number of potential tumour-suppressor genes were up-regulated in co-cultured T47-D cells including the calcium-binding protein S100A9 which showed an approximately 30-fold increase in the presence of MEC. S100A9 has been implicated in the control of cell growth, and therefore was a potential mediator of the MEC-induced reduction in proliferation. The elevated expression of S100A9 was approximately 3-fold, confirmed by real-time PCR. To investigate whether the MEC-mediated reduction in proliferation is mediated via S100A9, co-cultured T47-D cells were transfected with S100A9 siRNA and showed approximately 60% reduction in S100A9 expression compared to controls, using real-time PCR. Preliminary experiments showed no significant proliferation-suppressor effect of MEC in the presence of S100A9 knockdown compared with 12% suppression from control cells, and now is under further investigation. In conclusion, these initial data appear to indicate a role for S100A9 in the tumour-suppressor effect of MEC.

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Rapid Decalcification Of Total Laryngectomy Specimens

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We report a rapid decalcifying method for total laryngectomy specimens. This involves immersion of the larynx and attached tissues in an aqueous solution of 7% Nitric acid for up to 24 hours. After which serial sectioning can be undertaken with ease and allow processing of sledge blocks for preservation of the anatomy.

The current published guidelines on handling of laryngectomy specimens advocates serial sectioning of the larynx prior to decalcification. One such referenced method illustrates full cross sectioning of the larynx using a specialised slicing machine to gain 4mm sections. However, our method involves no specialist tools and can be carried out in every histopathology laboratory with minimal disruption.

We have found this method useful in dealing with these complex specimens and it also allows quicker and easier handling. The preservation of nuclear staining is comparable with our previous method and the ability to carry out immunohistochemistry on treated sections is also preserved.

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Expression of Cutaneous Fatty Acid Binding Protein in Head and Neck Squamous Carcinoma

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Cutaneous fatty acid binding protein (C-FABP) is implicated in keratinocyte differentiation and is overexpressed in some cutaneous squamous cell carcinomas and in the more aggressive prostatic adenocarcinomas. This study has investigated C-FABP expression in head and neck squamous epithelial neoplasia, particularly in relation to the grade and stage of carcinomas.
Tissue microarray (TMA) blocks were constructed from 100 primary squamous carcinoma, associated nodal metastases, adjacent normal and dysplastic squamous epithelium Immunocytochemical labelling was performed for C-FABP and Involucrin. In a subset of 30 representative carcinomas, the labelling in the TMA was compared with that in whole tissue sections.
C-FABP showed cytoplasmic and/or nuclear expression in over 90% of primary and metastatic carcinomas with more extensive expression in well-differentiated carcinomas. There was moderate or substantial agreement between TMA blocks and whole tissue sections for C-FABP and involucrin (weighted kappa values 0.59 and 0.75 respectively). In TMA sections C-FABP and involucrin showed a strong positive correlation (p <0.01) in normal and dysplastic epithelium, primary carcinomas and nodal metastases.
TMA is a reliable technique to investigate cellular and molecular alterations in head and neck squamous carcinomas. C-FABP expression is associated with keratinocyte differentiation and with tumour grade in these carcinomas.

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Case Report: Branchial Cyst Carcinoma

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We present the case of a 47 year old man who presented with a four month history of a left sided neck swelling. Clinical diagnosis of a branchial cleft cyst was made and the lesion was removed. Histology revealed a squamous lined cyst with normal squamous epithelium, dysplastic squamous epithelium and invasive squamous cell carcinoma. Full work-up of the patient has shown no primary lesion and he remains well.
The existence of branchial cyst carcinoma has been debated since it was first described in 1882. Whilst the vast majority of malignant squamous cell carcinomas in the neck are due to nodal metastases, we feel that branchial cyst carcinoma is a true entity, and that this case fulfils the criteria first laid down by Martin et al and later modified by Khafif et al for diagnosis of this neoplasm.

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Accuracy of frozen section in the diagnosis of liver mass lesions

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Intraoperative frozen section (FS) is well established in hepatobiliary surgery, however, there is little published data on its accuracy. This study was conducted to evaluate the diagnostic accuracy and reliability of FS diagnosis of liver lesions and to find out causes of errors and pitfalls if any. A consecuative series of 285 frozen sections of the liver performed between 1998 and 2004 from 173 patients were included. All frozen and corresponding paraffin sections were reviewed and the cases were evaluated according to matching of diagnosis between frozen and paraffin sections. In addition the quality of FS was assessed and causes of poor quality were evaluated. Final histological diagnosis was divided into positive (32%) and negative (68%) for malignancy. Four cases (2%) had to be deferred to await subsequent permanent paraffin section. There were one false positive and two false negative diagnoses. Sensitivity was 96.9% and specificity was 99.1% and the overall accuracy to determine the lesions was 95%. The cases were analysed to ascertain the origin of the difficulties, which comprised pathological misinterpretation, sampling error and technical imperfection. Biliary duct adenoma was the most common entity that was confused with malignant tumours. Our data are comparable with similar studies of tumours in other sites, and confirm that the frozen section is an accurate and reliable method for intraoperative diagnosis of suspected liver lesions.

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A Scoring System For Reporting Frozen Section of Hepatobiliary and Pancreatic Lesions

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Frozen section (FS) is used in hepatobiliary surgeries to confirm malignancy, to evaluate margins and to diagnose metastasis. However the decision is not always straight forward and in 2 – 7% of cases the diagnosis is deferred to paraffin. Methods: A scoring system to categorize FS is proposed consisting of 5 categories similar to that used in interpretation of breast FNA and core biopsies: F1 – inadequate, F2 – benign, F3 – suspicious probably benign, F4 – suspicious probably malignant, F5 – malignant. A consecutive series of 190 cases consisting of 60 cases of pancreas and 130 cases of liver frozen and paraffin sections were included in this study. All cases were reviewed and assessed independently by 2 pathologists using the proposed scoring system. Results: There was highly significant inter observer agreeability between the observers (Kappa = 0.8). Variations were mainly between the F3 and F4 categories. The main reasons for variations between observers were poor quality frozen sections, presence of dense inflammation and fibrosis and mucinous neoplasm's. Conclusions: Our results showed that this scoring system is reliable and consistent. The benefits of this scoring system are it provides simple standardized information, eliminates the category of ''deferred to paraffin'' and makes it easy to undertake comparisons and audit studies.

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Quantitative analysis of haemochromatosis associated histological features; the effect of environmental factors on disease expression

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Introduction: Hereditary Haemochromatosis (HH) is a prevalent genetic condition resulting in excessive iron storage in the liver. Elevated hepatic iron is associated with increased toxicity, and can result in progression of steatosis and fibrosis. Histological diagnosis is subjective and is affected by the pathologists experience and other factors. Quantitative image analysis is objective method. **Materials and methods:** In this study, 53 liver biopsies from patients with HH were investigated. The effect of sex, age at biopsy, weekly alcohol consumption, and genotype was investigated on subjective and quantitative values obtained for iron, fibrosis and steatosis. Two observers assessed histological grading and staging of parameters and the results from these were compared with quantitative values obtained by image analysis system (LEICA Qprodit). MIb-1 and p53 antibodies were used to detect an increased cellular proliferation and p53 expression, respectively. **Results**: Spearman's correlation co-efficient revealed significant positive correlations between current subjective and the quantitative method. Logistic and Multiple Regressive statistical techniques indicated 1. males are at increased likelihood of developing steatosis compared to females, 2. older age at biopsy increases the risk of having pathological fibrosis, and 3. higher levels of weekly alcohol intake may elevate fibrosis based on quantitative measurements. Finally, Spearman's correlation coefficient indicated significant positive correlation between MIB-1 and fibrosis. **Conclusion**: We conclude that quantitative technique is an accurate method for assessing steatosis and fibrosis in liver biopsy specimens. Patients at high risk of developing progressive liver disease include male gender, older age and high consumption of alcohol.

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Protective Effect Of Liver Support Preconditioning On Hepatic Ischemia-Reperfusion injury in a Rat Model.

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Ischemia and reperfusion (I/R) injury is a common complex inflammatory phenomenon encountered in medical practices. This work was designed to determine which is more injurious to liver tissue, ischemia or reperfusion? and to investigate the protective effect of ascorbic acid, Silymarin and DDB preconditioning on (I&R) injury in a rat model. Eighty rats were exposed to different periods of I/R operation with total time of 60 minutes. They were divided equally into 4 groups. One of them was control group while the others were exposed to 10; 20 and 30 minutes ischemia respectively. Each group was subdivided into 4 subgroups which were preconditioned with either no medication, Ascorbic acid, DDB or Silymarin respectively. Histopathological assessment for liver biopsy was done twice, after ischemia and reperfusion. Serum levels of malondialdehyde, ALT, AST and LDH were determined. The pathological results showed that I/R injury were time related. In mild ischemia, the pathological changes improved during the reperfusion periods while in sever ischemia the changes were irreversible and even worsen during the reperfusion period. Preconditioning with Ascorbic acid, DDB and Silymarin minimize the pathological changes in all periods of ischemia. The serum levels of malondialdehyde, ALT, AST and LDH increased in the post reperfusion samples and were time-related. Preconditioning with Ascorbic acid, DDB or Silymarin attenuated the hepatic I/R injury. However, Ascorbic acid showed the best results.

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FATTY CHANGE IN HEPATITIS C: STEATOHEPATITIS OR NOT?

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BACKGROUND AND AIM: Steatosis has been implicated as an independent risk factor for liver fibrosis, poor response to treatment and hepatocellular carcinoma in chronic Hepatitis C (HCV) infection. Obesity and alcohol are associated with more advanced fibrosis in HCV, but overt steatohepatitis is unusual. We performed a pilot histological study to investigate whether fibrosis evolves differently in HCV patients with and without steatosis.
METHODS: We selected patients with adequate biopsy material who had Ishak fibrosis score 2-6 and either no steatosis or score $\geq$2 (>33% hepatocyte) steatosis from a database of 233 biopsies[1]. The liver biopsies were blindly reviewed by two pathologists with respect to fibrosis, steatosis, hepatocyte ballooning and activated hepatic stellate cells (HSC) and Mallory bodies (MB) using immunohistochemistry for smooth-muscle actin and ubiquitin. Liver biopsies from patients without HCV infection served as controls.
RESULTS: Biopsies from 19 patients were included. We found no association between hepatocyte ballooning, HSC activation, and distribution of fibrosis (zone 3 pericellular v. portal) and the presence or absence of steatosis in the biopsy. MBs were small and infrequent, usually requiring ubiquitin for detection, and only found in biopsies with steatosis and $\geq$3 fibrosis
CONCLUSION: In HCV fibrosis may be pericellular in zone 3 whether or not there is steatosis. The full-blown picture of steatohepatitis was not seen in patients with moderate steatosis and fibrosis. MBs may form, but are small and require ubiquitin for detection.
1. Wyatt JI *et al.* Steatosis and fibrosis in patients with chronic hepatitis C. *J*

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Distinctive Pattern Of Liver Histology In Poorly Controlled Type I Diabetes

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We describe four patients with type I diabetes who presented with poor glycaemic control, increasing insulin requirements and grossly elevated serum transaminases. Liver histology in these patients showed a distinctive pattern resembling that seen in inherited forms of glycogen storage disease (GSD). Hepatocytes were markedly swollen with well defined round to polygonal plant-like cell membranes and, clear to pale eosinophilic faintly fibrillary cytoplasm in areas. Microvesicles of variable size were seen in occasional hepatocytes. There was dispersed diastase-labile glycogen positivity. Megamitochondria were prominent and many of the hepatocytes had glycogenated nuclei, especially within acinar zone 3 but neither Mallory's hyaline nor crystals were present. Mild resolving steatohepatitis with a mild panacinar macrovesicular steatosis was seen. The portal tracts showed minimal inflammatory infiltrate. There was no evidence for the accumulation of iron, copper or alpha-1-antitrypsin. Kupffer cells are essentially normal. Sinusoids were indistinct in areas of hepatocytomegaly.
Conclusion: The liver histological appearances of patients with type I diabetes mellitus, with stormy glycaemic control, are reminiscent of those seen in GSD; poor diabetic glycaemic control should be considered in the differential diagnosis when such histological features are present. In the absence of significant necroinflammation it is assumed that the transaminase elevations are a consequence of 'leaky' hepatocytes.

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Ductular Reaction in Early Post-transplant Liver Biopsies : Effects of Immunosuppression

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Background/Aims: Early biopsies from orthotopic liver transplant (OLT) patients frequently show a so called "ductular reaction". We have previously demonstrated a relationship with the severity of rejection but the true significance of such a reaction in OLT livers is uncertain. In this study, we have assessed the effect of immunosuppressive therapy on the degree of ductular reaction in early post-OLT liver biopsies.
Methods: Early post-transplanted liver biopsies (1 week ± 4 days) were retrieved from ninety-six liver transplanted patients between 1994 and 2002. Post-transplant immunosuppressive therapies – A- Standard combined triple: Azathioprine, Steroids, Cyclosporine or FK506; B- FK506; C- Mycophenolate mofetil (MMF) – given were obtained form a review of cases records. The magnitude of the ductular reaction in biopsies from these patients was assessed by a semi-quantitative scoring system and by evaluating ductular reaction density (duct count / 1 mm^2) using an image analysis system. Finally, the amount of ductular reaction was compared in patients receiving immunosuppressive therapies A, B and C to those not receiving the same regimens.
Results and conclusion: In early post-transplanted liver biopsies, the ductular reaction was significantly lower in patients receiving standard combined triple immunosuppressive therapy ($P < 0.05$), compared with FK506 or MMF alone. This effect appears to be independent of the degree of rejection suggesting that this phenomenon in the post OLT liver may be a drug-induced effect.

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Epithelial to Mesenchymal Transition in Chronic Cholestatic Liver Disease

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Intrahepatic bile ducts (IBD) are damaged or lost during a range of chronic cholestatic liver diseases (CCLD); this is often associated with a 'ductular reaction' and fibrosis. The precise relationship between these processes is not defined. In a previous study of CCLD it was found that biliary epithelial cells may undergo transition to a mesenchymal phenotype during CCLD since they express a marker of early epithelial to mesenchymal transition (EMT), S100A4. The present study was designed to substantiate the evidence for EMT in cells with a biliary epithelial phenotype by detecting additional proteins acquired during phenotypic transition.

Immunohistochemical investigation of liver tissue from CCLD showed that early EMT markers (S100A4, vimentin and MMP-2) were expressed in epithelium of small and medium-sized bile ducts and detected intracellular signal transduction (phospoSmad2/3) elicited by TGF-β. Pre-transplant liver and non-cholestatic liver disease biopsies were used as controls. Scanning laser confocal microscopy on immunofluorescence-labelled sections showed that early EMT markers co-localise with cytokeratin 19 in the ductular reaction.

This study confirms that, during CCLD, epithelial cells lining IBD and within the ductular reaction may undergo EMT and have the potential to become invasive activated fibroblasts. This process is associated with a response to local TGF-β.

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Biliary Indocyanine Green Excretion in an Isolated Perfused Liver Transplantation Model after Ischemia-Reperfusion

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Aims: Liver graft biliary dysfunction following ischemia-reperfusion (IR) injury is an important issue in liver transplantation (LT) units. We investigated the hepatic elimination kinetics of indocyanine green (ICG), an exclusively biliary excreted cholephilic dye, and functional and morphologic integrity of liver cells in a canine LT model following IR. **Methods:** Ethics Committee approved experimental protocol consisted to infuse the liver of 24 foxhounds with 5°C cold Ringer's solution through the hepatic artery and the portal vein, to remove and store it in a preservation medium at 5°C following cold ischemic storage for 2,6,8,10h, and then to transfer it to a re-perfusion circuit basin at 37°C for 2h. **Results**: Hepatic ICG uptake under normal conditions was 94±1% of the injected dose and 94±2% following ischemia up to 10h. Biliary ICG excretion was 2.5±0.5 mg ICG in the control group. After 2h of ischemia, ICG excretion decreased to 1.8±0.4 mg. After 6, 8, or 10h, ICG excretion was significantly reduced to 0.5±0.1, 0.2±0.1, and 0.1±0.1mg ($p<0.03$,$p<0.01$,$p<0.01$). Biliary ICG excretion was impaired by increasing the ischemia time, whereas hepatocellular ICG uptake and bile flow rate were not significantly reduced. Rapid increase of ALT and AST was found as early as 6h of ischemia. After 10h AST and ALT were significantly higher than controls ($p<0.01$). Hepatocytes showed a progressive increase of the bile canaliculus area and a membranous to cytoplasmatic staining with antibodies against cytokeratins of biliary type. **Conclusions**: We validated an LT animal model to study biliary excretion. Impairment of the biliary ICG excretion is associated with cholestatic changes of bile canaliculi. An increase of the cytokeratine staining may point to a damage that begins at an early stage of reperfusion despite prompt recovery of the bile flow.

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) Presenting In-Utero With Recurrent Nephromegaly and Oligohydramnios: Pathological Features

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD) usually presents in adulthood but may rarely present in the fetus. We report the histopathological features in two fetuses, one associated with second trimester oligohydramnios, with no known family history, which led to diagnosis of ADPKD.
Clinical Details: The first pregnancy was terminated at 20 weeks' gestation following antenatal ultrasound detection of bilateral enlarged hyperechogenic kidneys and severe oligohydramnios. Examination of the mother on the basis of the histopathological findings revealed bilateral polycystic kidneys. The subsequent pregnancy was terminated at 14 weeks' following the targeted ultrasound demonstration of bilateral fetal nephromegaly.
Pathological Findings: The non-dysmorphic 20-week fetus showed markedly enlarged, smooth, reniform kidneys with numerous small macroscopic cysts. Histological examination revealed loss of corticomedullary differentiation with numerous variably sized cysts consistent with both tubular and collecting duct origin, numerous glomerular cysts, and no evidence of dysplasia. The liver showed portal biliary dysgenesis. Fragmented kidneys from the 14-week fetus showed similar features. The diagnosis was ADPKD.
Conclusion: The triad of: 1) cysts of pan-nephron and collecting duct origin with 2) prominent glomerular cysts in 3) the absence of renal dysplasia, is virtually pathognomonic of ADPKD, even in the absence of a family history, provided there are no syndromic associations. The presence of hepatic ductal plate malformation does not exclude the diagnosis. Ultrasound detection of bilateral fetal nephromegaly and oligohydramnios may be due to ARPKD or ADPKD and exclusion of renal disease in the parents is indicated.

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Relationship Between Apparent Diffusion Coefficients and Histopathological Features of Paediatric Tumours with Diffusion-Weighted Magnetic Resonance Imaging.

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Introduction: Diffusion weighted magnetic resonance imaging (DW-MRI) with mapping of apparent diffusion coefficients (ADC) is used in brain imaging. It has potential for quantifying tissue cellularity and may therefore be useful in assessment of paediatric malignancies. To assess the feasibility of the technique in children we analysed 14 DW-MRI scans in 13 children with mass lesions in relation to histopathological features to determine whether high and low cellularity lesions may be distinguished by their ADC values.
Methods: 13 children had inversion recovery (STIR), contrast enhanced T1 gradient or spin echo (CE-T1) and standard DW sequences (b=0 and 1000 s/mm^2) and ADC maps were calculated. Histological specimens were subjectively categorised as high, intermediate or low cellularity according to established examples. The signal difference to noise ratio (SDNR) of all lesions was measured. Lesion pixel values from the central three slices of the ADC maps were analysed.
Results: There was no difference in lesion SDNR for ADC maps, STIR and CE-T1. SDNR was highest on ADC maps in 5/14 (36%) scans. The median ADC for high cellularity lesions was 0.83 (25/75 centiles=0.74/0.85) and low cellularity lesions 2.01 (25/75 centiles=1.43/2.45) x10^{-3} mm^2/s (Kruskal-Wallis test, Chi-Square=9.519; p=0.023).
Conclusion: DW-MRI with ADC mapping is feasible in imaging of paediatric mass lesions in the chest, abdomen and pelvis with standard clinical equipment. The distribution of ADC values within a mass lesion appears to be determined by its cellularity. Many paediatric malignancies represent highly cellular 'small round cell' neoplasms and such imaging may therefore allow more accurate interpretation of imaging findings in predicting tissue features.

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Auditing the Process of Histopathology Referrals – Experience in a Specialist Paediatric Centre

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Introduction: Histopathological referral is an integral part of NHS practice, and of increasing importance with development of specialist reporting and managed networks. Little is known regarding the quality of the referral process.
Methods: We audited 105 consecutive cases referred to a specialist paediatric centre including direct referrals from pathologists (usually for an expert opinion), and referrals requested by a clinical team as part of patient assessment. We assessed patient information and biopsy information supplied, pathological material submitted and problems encountered.
Results: Of 105 referrals, 82 (78%) were pathologist-initiated and 23 cases (22%) were clinician-requested. Patient identification was supplied with all and age provided in 100 (95.2%). Stained slides were sent in 90 (85.7%) and unstained slides, blocks or wet tissue in 52 (49.5%). Significant problems were encountered in 8 (7.6%) cases. Two referrals contained slides from the wrong patient, one had an incorrect date of birth and in 6 cases wrong or misleading clinical information required contacting the referring team before the case could be reported. Major postal delays or breakages were noted in 2 cases. A referral scoring system was devised in which an ideal referral achieved $\geq$13, $\geq$8 was the minimum acceptable for safe reporting. The average score was 10 overall, and 11 cases (10.5%) were unsatisfactory. 6 (7.3%) pathologist-initiated referrals versus 5 (21.7%) of clinician-led referrals fell below the minimum level.
Conclusion: 10% of referrals contained insufficient material or information to allow accurate reporting. Clinician-led, rather than pathologist-led, referrals were associated with worse provision of information. A standardised histopathology referral process may be improve this process.

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Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) Findings in Relation to Histological Features in Paediatric Tumours: A Preliminary Study

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Introduction**:** FDG-PET scanning is an increasingly important imaging technique in adult oncological practice. However, there is little data regarding the role in paediatric oncology with limited experience of FDG-PET scanning in children. By comparing the FDG-PET scan findings with the pathological features of resected specimens, validity of FDG-PET scanning in common childhood cancers can be determined. We present four cases – two with neuroblastoma, two with Wilms tumour, to illustrate the importance of adequate imaging-pathological correlation in correct interpretation of new imaging techniques.
Method: All children received induction chemotherapy as per protocol and underwent standard ^{18}F-fluorodeoxyglucose PET scanning immediately prior to their surgery. Macroscopic and microscopic pathological findings of the surgical specimen were then compared with the FDG-PET study findings.
Results**:** All FDG-PET scans were positive. When compared with the pathological findings, three were true positives, representing viable tumour. Two had a focally positive area of increased FDG uptake correlating with areas of residual active tumour; areas of necrosis appearing negative on scan. One had diffuse FDG uptake correlating with diffuse viable tumour (stage 4 Wilms tumour). One scan however, was 'false' positive as there was no residual tumour identified pathologically but post-chemotherapy tumour areas demonstrated florid fibroblastic proliferation and macrophage infiltration rather than bland necrosis (stage 4 MYCN amplified neuroblastoma).
Conclusion**:** FDG-PET scanning is a useful guide to tumour status after induction chemotherapy. However, results must be interpreted with caution since florid reactive proliferations postchemotherapy may also appear PET positive.

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Myogenin And Desmin Immunohistochemistry In The Assessment Of Postchemotherapy Genitourinary Embryonal Rhabdomyosarcoma: Prognostic And Management Implications

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Introduction: Post-treatment genitourinary embryonal rhabdomyosarcoma (GUeRMS) often exhibits well-differentiated rhabdomyoblasts detectable on H&E staining. Definite areas of persistent undifferentiated RMS indicates residual/recurrent disease. However, increased use of surveillance biopsies and immunohistochemical stains may reveal scanty immunopositive cells in biopsies which appear bland on light microscopy, the clinical significance of which is unknown.
Methods: Postchemotherapy follow-up biopsies from 14 children, aged 8m-7y (median 2.8years), with GUeRMS, (10 bladder, 2 vaginal) were immunostained with Desmin and Myogenin and examined in relation to clinical outcome.
Results: Two patients had obvious residual eRMS on routine H&E staining and were excluded from further analysis. In 12, no obvious residual eRMS was present following initial therapy but 11 of these demonstrated presence of well-differentiated large rhabdomyoblasts, and one showed no morphological evidence of any RMS. All 12 cases however demonstrated scattered Desmin and/or Myogenin-positive small bland cells. Four were managed conservatively, eight had further treatment. One patient died from disease but all others remain alive and well 8m-13y post-treatment, regardless of immunohistochemical findings.
Conclusion: The presence of scattered Myogenin/Desmin-positive cells in post-chemotherapy GUeRMS specimens in the absence of morphologic evidence of residual disease on routine H&E staining does not provide clinically useful prognostic information since such cells can be detected in all cases and their presence does not appear to influence outcome.

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Paediatric Cardiac Chondroma With Superior Vena Cava Obstruction: Pathological Findings

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Introduction: Histologically benign visceral chondromas are extremely rare in childhood, except the lung, where they may represent a component of Carney's syndrome. Primary cardiac chondromas are exceptionally rare in patients of any age, with only two cases previously reported. We present a case of a teenage boy with a huge primary intracardiac chondroma.
Methods: A 16 year old boy presented with severely reduced exercise tolerance and dyspnoea. Imaging demonstrated a huge lobular mediastinal mass with focal calcification causing superior caval obstruction and hemodynamic compromise. Thoracotomy demonstrated a tumor arising from the atrial septum. The tumour was resected on cardiopulmonary bypass, and the heart reconstructed. The tumour was smooth, white, multi-lobulated, and solid, 952 gm, 16cm maximum diameter. The cut surface demonstrated a multi-lobulated glistening white, predominantly solid appearance, areas of cystic degeneration. Multiple sections revealed nodules of bland hyaline cartilage, with pushing margins with no cytological atypia and no spindle cell, or other hypercellular, areas present. The histopathological features were those of a benign chondroma.
Discussion: Primary cardiac extraskeletal chondroma is exceedingly rare, this being only the third case reported. However, other tumours with chondroid differentiation may be seen in the heart including metastatic extraskeletal myxoid or mesenchymal chondrosarcoma, primary cardiac chondrosarcoma, primary atrial myxofibrosarcoma and focal chondroid differentiation in otherwise typical primary benign atrial myxomas.
Conclusion: Primary benign intracardiac chondroma may occur in the paediatric population, in association with venous obstruction.

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Clinicopathological Features of Wilms Tumour with Persistent Intravascular Extension

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AIM: Review of clinicopathological features of Wilms tumour with intravascular (inferior vena cava and/or atrial) tumour extension.
PATIENTS: Data was collected regarding operative details, tumour and 'thrombus' histology, and outcome for patients with Wilms tumour with intravascular tumour extension.
CLINICAL DETAILS: From 1988 - 2004, 13 patients were identified who required surgery for Wilms tumour with persistent intravascular extension. The mean age was 56 months (range 31 - 108 months). Pre-operative chemotherapy (mean 10 weeks; range 1 - 29 weeks) was administered in 11/13 patients. The intravascular extension of tumour was infra-diaphragmatic IVC in 5, supra-diaphragmatic IVC in 1, and right atrial in 7 patients. In addition to nephrectomy (all patients), excision of the intravascular component was performed with local cavotomy, extensive cavotomy without cardio-pulmonary bypass (CPB), and excision of cavoatrial tumour with CPB with caval repair. There were no intra-operative deaths.
HISTOLOGICAL FINDINGS. Histology of the renal tumour was favourable in 11 and unfavourable in 2. The intravascular tumour component excised was composed of viable Wilms tumour in 10 (77%), all with superimposed surface thrombus. The remaining cases demonstrated extensive postchemotherapy changes and propagating benign thrombus only, with no viable malignant cells in the intravascular component.
OUTCOME. Mean follow-up at 59 months (range 2 - 180) revealed 3 deaths from disease, 3 clinical relapses (none within the vena cava) and 7 remaining disease-free.
CONCLUSION: Wilms tumour with persistent intravascular extension is often associated with persistence of viable malignant intravascular tumour but may be amenable to surgical excision.

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Blood Film Examination for Detection of Vacuolated Lymphocytes: Experience of >2,500 Cases from a Single Paediatric Pathology Centre

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Introduction: A range of metabolic diseases may result in abnormal accumulation of metabolic by-products resulting in abnormal lymphocyte cytoplasmic vacuolation, identifiable on routine blood-film examination. This study retrospectively examines the use of blood-film examination for vacuolated lymphocytes in a specialist Paediatric Pathology Department in relation to patient age and presentation.
Methods: Retrospective review of a Histopathology database to identify all blood films examined for detection of vacuolated lymphocytes during a 15-year period (1989-1994).
Results: 2,550 blood films were included, the median age at submission being two years (Range=birth-88 years), with >90% from children ≤18 years'. The commonest indications were developmental delay/regression, ataxia, seizures and cardiomyopathy. Vacuolated lymphocytes were identified in 156 films (6.1%). The frequency of vacuolated lymphocytes varied with clinical presentation, opthalmic indications having the highest positive rate (40%). In cases with vacuolated lymphocytes present, a wide range of underlying metabolic diagnoses were apparent, the commonest being juvenile neuronal ceroid lipofuscinosis and acid-maltase deficiency, which accounted for around half of the diagnoses. Others included, GM1-gangliosidosis, galactosialidosis, Salla disease, Mannosidosis, I-cell disease and others.
Conclusions: Routine examination of blood-films for lymphocyte vacuolation is clinically useful in patients with history suggestive of metabolic disease. The test is cheap, rapid, minimally invasive and provides a first-line screening test with some findings providing specific clues to the underlying diagnosis.

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Paediatric Testicular and Paratesticular Pathology: Histopathological Spectrum and Relative Distribution

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Introduction: A wide range of testicular pathologies may be encountered in the paediatric population, many uncommon in adult practice. The aim of this study was to determine the histopathological spectrum and relative frequencies of such entities from a specialist paediatric centre.
Methods: A search of a computerised histopathology reporting database was carried out to identify all cases of testicular pathology during a 15 year period (1989-2004).
Results: During the period 551 paediatric testicular and paratesticular specimens were examined. The median age was 3 (range 0-16) years. The distribution of diagnostic categories was as follows: Vanished testis / testicular regression 117 (21.3%), cryptorchidism 105 (19.0%), torsion 96 (17.4%), tumour 95 (17.3%), intersex 89 (16.2%), non-specific description only 15 (2.7%), hernial sac 9 (1.6%), hydatid of Morgagni 9 (1.6%), miscellaneous 8 (1.5%), ectopic adrenal tissue 3 (0.5%), epididymo-orchitis 3 (0.5%) and inadequate sample 2 (0.4%). Within the 'tumour' group, 49 (52%) were primary testicular / paratesticular lesions whilst 46 (48%) were metastatic involvement of the testis, almost always in association with acute lymphoblastic leukaemia / lymphoma. Primary tumours were predominantly teratomas, yolk sac tumours, juvenile granulosa cell tumours and paratesticular embryonal rhabdomyosarcoma.
Conclusion: The spectrum of histopathological entities affecting the testis in childhood includes predominantly cryptorchidism, vanished testis, intersex-related disorders, torsion and tumour. The range and distribution of both benign and malignant entities encountered is markedly different from that seen in adult testicular surgical pathology practice.

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Immature Testicular Germ Cells Detected Using Placental Alkaline Phosphatase (PLAP) and c-Kit (CD117) Immunostaining in Relation to Risk of Germ Cell Malignancy in Children with XY Intersex and Isolated Cryptorchidism

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Introduction: The risk of subsequent development of testicular germ cell neoplasia is related to underlying developmental defects such as uncomplicated cryptorchidism, in which the risk is around 0.5% and XY intersex with abdominal testes, in which the risk may be as high as 20-25%. Detection of ITGN in paediatric testes using PLAP and CD117 immunohistochemistry is difficult since immature germ cells may retain such expression. The relation between the increased risk of malignancy and prevalence of immature germ cells expressing PLAP or CD117 is unknown.
Methods: Archival cases of uncomplicated cryptorchidism (CO) and XY intersex (INT) were identified, anonymised and sections immunostained with PLAP and CD117, the expression of which was semiquantitively recorded. Expression was compared between groups. The study had LREC approval.
Results: 58 cases were examined, median age 3 (range birth-11) years, including 39 CO and 19 INT. There was no significant difference in age distributions between the groups (P=0.15) The prevalence of any PLAP+ germ cells was 2/39 (5.1%) versus 3/19 (15.7%) respectively. (z=-1.4, P=0.17). In the PLAP+ cases, the median number of PLAP+ germ cells per tubule was 1.7, which was similar in both groups. In contrast, 94% of cases showed presence of any CD117+ germ cells. The frequency of CD117+ cells was not significantly different between groups (P=0.27)
Conclusion: CD117 and PLAP identify different populations of germ cells in paediatric testes. The higher risk of malignancy in XY INT does not appear to be simply related to increased numbers of immature PLAP+ / CD117+ germ cells present.

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Medium-Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency Presenting as Early Neonatal Death in Association with Maternal Gestational Diabetes Mellitus

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Introduction: Medium-Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency is one of the more common paediatric metabolic disorders which may present with sudden unexpected death. In the majority of cases this occurs in infancy or early childhood, often in association with a coexisting infectious episode. We present a case of early neonatal death of a child with MCAD in whom the atypical presentation may have been a consequence of maternal gestational diabetes mellitus.
Case Report: The mother was in her second pregnancy, throughout which she had declined all hospital-based antenatal care and screening tests. She had a midwife-attended home birth following spontaneous labour at term. The delivery was apparently uneventful, resulting in a 4.3kg baby girl. On day 2 of life, she was noted to be feeding poorly, collapsed and died. Postmortem examination was unremarkable but routine histological examination according to protocol revealed apparent islet cell hyperplasia consistent with infant of a diabetic mother. Tandem mass spectrometry of blood and bile suggested MCAD, which was confirmed by genetic testing, the infant possessing the 985A>G Exon11 *Nco*1 mutation. We propose that the combination of neonatal hyperinsulinism, secondary to probable maternal gestational diabetes, and underlying MCAD, resulted in severe hypoglycaemia and death in the early neonatal period.
Conclusion: The presentation of genetic metabolic disorders such as MCAD may be modified by other abnormalities of metabolic control and underlying defects, such as MCAD, should be considered in all neonatal deaths even though such presentation in classically atypical.

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Infantile ALK-Expressing Anaplastic Large Cell Lymphoma With t(2;5) Translocation

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Introduction: Anaplastic large cell lymphoma (ALCL) accounts for 10-30% of NHL in childhood, the average age at diagnosis being 8-10 years with a reported range of 1 to 15 years. We present a case of ALCL affecting a five-month old infant.
Case Report: The patient was a five month-old child with no significant family history and an uncomplicated prenatal and postnatal course. He presented with fever, multiple cutaneous nodular lesions affecting extremities and localized lymphadenopathy. Skin biopsy demonstrated normal epidermis with a florid dermal cellular infiltrate composed of large cells with clear cytoplasm and rounded or reniform nuclei. Immunohistochemical staining demonstrated the large infiltrating cells to express membranous and Golgi CD30, CD3, EMA and CD15, with nuclear and cytoplasmic staining with ALK1. RNA was extracted and RT-PCR carried out, which demonstrated a t(2;5)(p23;q35) translocation which results in production of the NPM-ALK fusion transcript. He was treated according to the FAB LMB 96 protocol, regimen B, had an initial good response, but relapsed and received salvage chemotherapy with an apparent complete response and was in continued remission when last seen four months later.
Discussion: We have reported a case of an infant with ALCL, demonstrating nuclear and cytoplasmic immunohistochemical ALK1 staining and presence of the t(2;5)(p23;q35) translocation resulting in production of the NPM-ALK fusion transcript. This is the youngest case of such ALCL thus far described. Diagnosis is aided by the immunohistochemical expression of ALK, which has been reported in the vast majority of paediatric cases of ALCL.

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The Histopathology of Testicular Regression Syndrome ("Vanished testis"): 117 Consecutive Cases from a Paediatric Pathology Department

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Introduction: Testicular regression syndrome (TRS) or 'vanished testis' (VT) are terms used for the condition in which no testicular tissue can be identified following exploration for clinical cryptorchidism. A spectrum of pathological findings may be present but there is little literature systematically examining the histopathological features.
Methods: Search of a computerised paediatric histopathology reporting database was performed to identify cases of TRS / VT. Cases were anonymised, basic demographic data obtained and the histopathological findings reviewed.
Results: 117 cases were identified (1989-2004), the median age was 2 (range birth-12) years. In 52 (44%) a nodule was identified macroscopically, median maximum diameter 0.5 (range 0.1-2.0) cm. Microscopic haemosiderin laden macrophages were present in 32 (27%), dystrophic calcification in 52 (44%), residual testicular tubules in 14 (12%), vas deferens in 71 (61%), epididymal tissue in 39 (33%) and prominent spermatic cord vessels in 43 (37%). The likelihood of finding a macroscopic nodule and haemosiderin laden macrophages reduced with increasing patient age (t-2.1, P=0.04 and t=-2.5, P=0.01 respectively), whereas there was no significant relationship between age and likelihood of finding histological testicular or vas deferens remnants.
Conclusion: There is a histopathological spectrum of changes associated with the clinical condition of TRS / VT, including presence of a fibrous nodule, with or without testicular, epididymal or vas remnants, haemosiderin-laden macrophages and calcification. The histological findings are partially related to patient age, consistent with the hypotheses of prenatal torsion / trauma as an underlying aetiology.

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Inadequacy Rates And Optimal Processing Of Rectal Suction And Punch Biopsies For Suspected Hirschsprung's Disease

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Background: In our centre, a high rate of inadequacy in rectal suction and punch biopsies was causing diagnostic difficulties, and processing did not always conform to published gold standards.

Methods: A computer search for frozen rectal biopsies over a 2 year period found 134 cases including 290 biopsies. The following items were recorded: patient demographics, number of biopsies, biopsy diameter, adequacy, presence of squamous mucosa, AChE pattern, number of serials cut and the first section on which ganglion cells were present. Extra work was performed if ganglion cells were absent.

Results: 205 biopsies were inadequate. In 51% of cases at least one biopsy was adequate, while all biopsies were inadequate in 49% of cases. Older age was a significant factor in causing inadequacy. In adequate biopsies with a normal AChE pattern, ganglion cells were identified in 95% of cases after 36 serials, and in 100% after 60 serials. In all biopsies, where ganglion cells were not initially identified after 36 serial sections, performing extra serials revealed them in 50% of cases, and processing frozen material to paraffin revealed them in 46% of cases.

Conclusion: 36 serial sections suffices for 95% of biopsies, but the "gold standard" of 60 serials is validated.

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Microarray Comparative Genomic Hybridisation Analysis in Cardiac Rhabdomyoma- A Case Study.

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Primary paediatric cardiac tumours are rare, with an estimated incidence of 0.27 % at autopsy with the most common being, rhabdomyoma. Cardiac rhabdomyoma is associated with tuberous sclerosis in 90 % of cases, however the mechanisms underlying this association are not well understood. In this study we examine the genomic aberrations in an individual case of cardiac rhabdomyoma using microarray based comparative genomic hybridisation technology. T
An autopsy was carried out on a female infant who died in utero at 36 weeks gestation. A provisional clinical diagnosis of Tuberous Sclerosis (TS) was suggested on the basis of a cardiac tumour identified by intra-uterine fetal scan. Specimens were obtained at autopsy from the cardiac tumour, normal lung tissue, and a blood specimen. Histology confirmed the presence of a cardiac rhabdomyoma, the autopsy found no evidence of TS.
Genomic DNA extracted from tumour and normal tissue was labelled by random priming with Cyanine 3 dCTP and Cyanine 5 dCTP, respectively, and co-hybridised to the Genosensor Array 300 CGH array from Vysis. Dye reversal experiments were also performed to validate the results.
The array CGH profile of the cardiac rhabdomyoma showed gains at loci within chromosomes 1, 2 4, 5, 7, 9, 11, 12, 13 and 18, which reflect genetic aberrations within the tumour. Of these amplifications, 50% occurred at sub-telomeric loci. Of particular interest, is an amplification of region 5q11.2-q13.2, which codes for the DNA mismatch repair gene MSH-3. Other amplified targets included regions encoding the oncogenes FGR, EGFR, GLI and YES1. The data describes underlying genetic abnormalities that are potentially important in the development of this tumour.

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Loss of Chromosome 22q in an infantile Rhabdoid Tumour of the Liver

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Aims: Rhabdoid tumours are highly malignant tumours with rhabdoid cells and can be located at both renal and extrarenal sites. Treatment includes chemotherapy and frequently surgery. We report on a 1-year old child with a rhabdoid tumour of the liver. The child was treated by chemotherapy and partial hepatectomy following no response to the medical treatment.
Methods: Immunohistochemistry and comparative genomic hybridization (CGH) were performed.
Results: We found a high malignant rhabdoid tumour of the liver with pronounced infiltration of the liver parenchyma without formation of a pseudocapsule. Immunohistochemistry revealed a positivity of the tumour cells for pancytokeratin, cytokeratin 8, (focal) vimentin, epithelial membrane antigen, CD34 and CD99. The tumour was negative for cytokeratin 7 and 19 and beta-catenin as well as for alpha-fetoprotein, c-kit, S-100 protein, chromogranin A, synaptophysin and CD56. Ki67 showed at least 50% of tumour cells in a proliferative phase. CGH showed loss of 22q chromosome.
Conclusions: Loss of 22q and 19 have been described in rhabdoid tumours of the brain. To the best of our knowledge this is the first youngest case of rhabdoid tumour of the liver showing loss of 22q.

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Expression of Laminin, Tenascin and Collagen IV in the Developing Ductal Plate of Normal Foetuses and Foetuses affected with Trisomies 13, 18 and 21

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Aims: Intrahepatic bile duct development can be classified into three distinct stages: ductal plate, remodelling ductal plate, and remodelled bile ducts. Collagen IV, laminin, and tenascin have been proposed to interplay in the epithelium-mesenchyme interaction. We studied their expression during bile duct development in fetuses with abnormal and normal karyotype.
Methods: Fetuses with *normal karyotype and no anomalies* (n=9), *trisomy 13* (n=8), *trisomy 18* (n=9), *trisomy 21* (n=10) were included in this study. Avidin-Biotin-Complex immunohistochemistry method was used. Ethics Committees approved the project.
Results: Collagen IV was observed throughout the ductal plate development and in each of the four groups, although the intensity of the expression varied. The strongest levels of expression were observed in the normal group, whilst the weakest levels of expression in trisomy 13. Laminin was observed in every group and at each stage. The strongest intensity of expression was observed within the normal and trisomy 18 groups, whilst the weakest intensity of expression in liver sections from fetuses with trisomy 13. Tenascin was expressed weakly and irregularly.
Conclusions: Mesenchymal markers expression during the development of ductal plate shows variability in foetuses with chromosomal imbalances.

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Epigastric Heteropagus Twins (EHT): Anatomo-Pathological Study of a Case

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Aims: EHT are an exceedingly rare form of asymmetric conjoined twins in whom the dependent part (parasite) is attached to the epigastrium of the dominant part (autosite). In 2000 Özcan et al. reviewed the English-literature and found only thirteen well-described EHT cases and added one case of their own. We report an additional case of asymmetric twinning of epigastric type.
Methods: A 1-day-old female EHT was promptly diagnosed and surgically resected.
Results: The malformation consisted of a well-developed autosite and a parasite attached at the level of the epigastrium of the autosite. The parasite consisted of two arms, an abnormal trunk, a small abdomen, a pelvis, and two well-formed legs. There were female genitalia, but atresia of the urethra and anus. Vascular study showed a vascular supply from the internal mammary artery of the autosite. Pathology study showed no limb musculature and no innervation. There were also a multicystic kidney dysplasia and multiple bowel atresia. Twelve months follow-up was successful.
Discussion: The most probable pathogenesis is an incomplete cleavage of the embryo at the 2nd week of gestation. Surgery may be a successful option.

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Do Placentas Submitted For Histopathology At A Teaching Hospital Fulfil Best Practice Guidelines For Examination?

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Following publication of ACP best practice guidelines for placental histopathological examination (2004), we have audited our placenta workload using these guidelines as the gold standard.
All placentas submitted between May and September in one calendar year were included in the study. In all cases, clinical details provided, number of blocks taken and final diagnoses were recorded. The specimens were retrospectively categorised according to ACP guidelines, and potential new block totals calculated.
155 specimens were received during this period. Total block numbers were 581. 17% of specimens had insufficient clinical details to allow categorisation within the ACP groupings.
Using the guidelines, 66% would have required examination macro and microscopically, 15% macroscopically only and19% would have been stored only.
Adoption of ACP guidelines would have reduced specimen numbers examined microscopically by 30%, and blocks processed by around 20%.
The histological findings from all specimens were analysed. Significant diagnoses (e.g. chorioamnionitis) were found more commonly in the microscopic examination group, but were also found in macroscopic only and storage groups.
We have shown that by adopting ACP guidelines, with more detailed examination of fewer specimens, there would be an overall reduction of our workload. However, adoption of the guidelines would require provision of more accurate and complete clinical details on request forms.

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Two cases of proptosis with sphenoid wing expansion: Intraosseus Meningioma and Meningioma en plaque

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PURPOSE: Two patients presented with proptosis due to rare intraosseous meningioma. The clinical, radiological and histological findings are presented. The differential diagnoses are discussed and the literature is reviewed.
BACKGROUND: Primary intraosseous meningioma of the skull is a rarely encountered lesion often confused preoperatively with a primary bone tumour of the skull.
HISTORY: Two patients presented with proptosis and radiological findings of an intraosseous expansile growth. In both cases computed tomography (CT) showed expansion of the right sphenoid wing by a sclerotic lesion. In one of the cases the lesion was entirely intraosseous, in the other case there was an additional intracranial soft tissue component identified.
OUTCOME: Bone biopsy revealed intraosseous meningioma. One lesion was a primary entirely intraosseous meningioma, the other lesion has to be regarded as meningioma en plaque (MEP) with intraosseous extension.
THERAPY: Conservative treatment was chosen in the absence of any other problems but proptosis. In general the treatment of choice is wide surgical resection, where practical, as for meningiomas located in the intracranial cavity, followed by cranial reconstruction.
CONCLUSIONS: Intraosseous meningioma is a benign rare tumour of slow progression, which can present with painless proptosis. The CT scan provided differential diagnosis including fibrous dysplasia, Paget's disease and intraosseous meningioma. The literature suggests that MEP and primary intraoss. mening. share more similarities than differences and make differentiation between both entities difficult and clinically negligible.

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Angiotropic Lymphoma: Four Cases With Very Different Presentations

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Angiotropic Lymphoma, synonymous with Intravascular Large Cell Lymphoma and Malignant Angioendotheliomatosis, is a rare, aggressive non-Hodgkin's lymphoma. The clinical presentation can be extremely variable, making antemortem diagnosis difficult. We report four cases of angiotropic lymphoma diagnosed at autopsy.
The first patient presented with non-specific symptoms including fever, rigor and cough. The second patient presented with pyrexia of unknown origin, the third patient, who had a history of orbital non-Hodgkin's lymphoma, presented with dizziness, fainting and shortness of breath and the fourth patient presented with non-specific neurological symptoms.
In all four cases, microscopy showed intravascular clusters of anaplastic lymphoid cells within the brain. These cells which had pleomorphic nuclei, disordered chromatin and prominent nucleoli markedly distended and obliterated the vascular lumina. The cells stained positively with CD20 (B-cell marker), CD45 (pan-lymphoid marker) and negatively with CAM 5.2 (epithelial marker).
Angiotropic Lymphoma usually affects older patients, but has no predilection for either sex. Because of its multisystem involvement, the clinical presentation can be highly variable.
The purpose of this report is to highlight the wide range of clinical presentations of this disease and to emphasise the importance of including it in the differential diagnoses, especially in cases of pyrexia of unknown origin.

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Utility of the Immunohistochemical Detection of FLI-1 Expression in CNS PNET (Medulloblastoma) Using a Monoclonal Antibody

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Background: FLI-1 is a member of ETS genes, which encodes for a family of transcription factors, defined by a highly conserved DNA- binding domain. Rossi et al, in their study, raised a novel monoclonal antibody against the carboxyl terminal of the FLI-1 protein and tested it in a series of small round cell and vascular neoplasm. They showed a strong FLI-1 immunoreactivity in all Ewing's Sarcoma/PNET (peripheral PNETs).
Aims: the aim of this study is to use the same immunohistochemical staining method on CNS PNETs (Medulloblastona) and to see whether the central and peripheral PNETs show the same immunoreactivity to FLI-1 antibody.
Methods: the monoclonal antibody against the FLI-1 protein was used to stain the Formalin-fixed, paraffin-embedded archival tissue from 16 Medulloblastoma, 7 Ewing's Sarcoma and 3 Neuroblastoma. Positivity was scored using a four tiered scale (1+ = <25% positive cells, 2+ = 26- 50%, 3+ =51- 75%, 4+ =76- 100%).
Result: 7/7 (100%) of Ewing's Sarcoma exhibited 4+ strong immunoreactivity, whereas just 4/16 (25%) of Medulloblastoma showed 4+ immunoreactivity. All 3 Neuroblastoma were negative for FLI-1.
Conclusion: The results indicate that CNS PNETs and peripheral PNETs does not show the same FLI-1 gene expression and FLI-1 antibody immunostain can not be used for detection of CNS PNETs.

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Primary Meningeal Lymphoma - A Case Report

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Primary central nervous system lymphoma is an invasive disease in both HIV-positive and HIV-negative patients. Atypical presentations, including meningeal involvement, have been reported but primary meningeal B-cell lymphoma appears to be very rare.
Primary meningeal lymphoma is a rare disorder, and the neuroradiological characteristics or the complication of this rare disorder have not been well reported. We hereby present 2 patients with primary meningeal lymphoma. The first patient is a 70 years old female who presented with confusion and collapse and a left parafalcine lesion on CT imaging. The second patient is a 74 years old female who was admitted with right hemiparesis and seizure and her CT scan showed a large left fronto-temporal lesion. The microscopic examination of the tumours from both patients showed a cellular tumour with an attached band of fibrocollagenous tissue (consistent with dura). The tumour cells were arranged in a diffuse sheet-like pattern. The cells were uniform, small, round with rounded nuclei, scanty cytoplasm and condensed chromatin. Both tumours were limited to the fibrocollagenous tissue (meninges) and there was no diffuse involvement of the underlying brain parenchyma. The immunohistochemistry of both tumours confirmed the diagnosis of B cell lymphoma.

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Pathogenetic Mechanisms In Multiple Sclerosis: unity or diversity?

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Multiple Sclerosis (MS) is pathologically fairly uniform. Recently, it was suggested on the basis of immunohistological findings, that 4 different types of pathogenetic mechanisms might underlie MS: 1) a T-cell/macrophage mediated type, 2) a antibody/complement mediated type, 3) an oligodendrocytopathy with apoptosis of the myelin-forming oligodendrocytes, and 4) a process involving non-apoptotic cell death of oligodendrocytes and unusal sequential disappearance of myelin proteins. This was based on material that was not exactly representative for relapsing-remitting MS, with a high percentage of biopsy cases and of cases with a very short clinical course. Therefore, this needed verification on a large, more conventional material.

We studied actively demyelinating lesions of 30 patients for the presence of T- and B-cells, Complement proteins, activated caspase 3 and morphological signs of apoptosis, myelin proteins and myelin degradation to assess what disease mechanisms.

Results show: there were few T-cells in the lesional parenchyma itself; all lesions showed complement depositions; signs of apoptosis are rare inside and outside of the lesions; in some lesions oligodendrocytes are abundant, while in others they tend to disappear, non-apoptotically.

Pathogenetically our conventional material is fairly uniform, with type 2 mechanisms predominating. Possible explanations are discussed.

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Identification of a novel gene, REO1, on chromosome 1p36.32 that shows reduced expression in oligodendrogliomas

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Loss of chromosome arms 1p and 19q is hallmark of oligodendrogliomas, suggesting that these chromosomes carry critical tumor suppressor genes. To isolate the target genes on chromosome 1p, we have mapped 2 minimally deleted regions on 1p36.31-36.32, in which 8 genes are annotated. One candidate gene from the deletion regions showed absence of or reduced expression in 8 of 13 microdissected oligodendrogliomas and is thus named Reduced Expression in Oligodendroglioma 1 (REO1). Herein, we reported the molecular findings of REO1 in a cohort of 38 WHO grade II and 25 WHO grade III oligodendrogliomas. Real-time RT-PCR showed that 34 of 63 (53%) tumors had reduced REO1 expression, with 19 of them showing more than 10-fold reduction when compared to that of normal brain tissues. No somatic mutations of REO1 were detected. We then evaluated promoter hypermethylation as a possible mechanism for reduced REO1 expression. Our preliminary data on 20 samples revealed that 11 of 13 tumors with reduced REO1 expression showed dense promoter hypermethylation, whereas 5 of 5 tumors with normal expression levels and 2 normal brain tissues showed scarce or no aberrant hypermethylation. Taken together, our results demonstrate that reduced expression of REO1 is involved in oligodendrogliomas and such expression downregulation may be attributed to promoter hypermethylation.

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Spectrum of Histological Changes in Fuch's Endothelial Dystrophy

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Aim: To investigate the spectrum of histological changes seen in corneal buttons with a clinical diagnosis of Fuch's Endothelial Dystrophy.
Method: A retrospective study was undertaken to review histology slides of patients who underwent penetrating keratoplasty for corneal decompensation secondary to clinical suspicion of Fuch's dystrophy. Main outcome measures included changes seen in epithelial layer (basal intraepithelial oedema, epithelial bullae, subepithelial fibrous membrane), stroma (stromal thickness), descemet's membrane (thickness, presence of prominent and buried warts, lamination), and endothelial layer (reduction in number of cells, presence of cytoplasmic vacuoles and pigments).
Results: A total of 20 cases were identified and included in the case study. The mean age of patients was 72.4 ± 9.5 years and 65% of patients were female. No histological evidence was found in 20% of clinically diagnosed FED. Of histologically confirmed cases of FED, endothelial assessment was not possible in 18.7% of cases because of artefactual loss of endothelium. Where assessment of all 4 layers was possible, 100% of cases showed changes in each layer although with a variable range.
Conclusion: The diagnoses of FED cannot be made on the presence of corneal guttata and endothelial pigment alone and a positive diagnosis requires accompanying epithelial and stromal oedema

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Basal Cell Carcinoma Extensively Colonized By Benign Melanocytes

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Introduction:
We report the case of a pigmented basal cell carcinoma (BCC) clinically masquerading as a malignant melanoma (MM) and histologically extensively colonized by benign melanocytes.
Case report:
An 82 year old Caucasian female presented with a bluish-black cutaneous nodule on her right thigh that had been increasing in size over the last one year. Clinical opinion favoured a MM. Macroscopic examination showed a skin ellipse 70 x 35 x 20mm with a well-defined 30mm diameter bluish-black surface nodule. Microscopy revealed a pigmented BCC colonized by numerous HMB45 positive melanocytes, some containing pigment and numerous pigment laden macrophages present throughout the tumour lobules as well as in the surrounding stroma.
Discussion:
Several non-melanocytic pigmented epithelial neoplasms of the skin can be confused with MM, particularly pigmented BCC. BCC can unusually coexist with benign or malignant melanocytic lesions. The presence of HMB45 positive melanocytes within BCC is only described in a few papers in the literature. Our case shows that a pigmented BCC can be populated by an abundance of melanocytes with a consequent potential for misinterpretation as to the significance. We suspect that melanocytes are common in pigmented BCCs and are also a component of non-pigmented BCCs but their presence has not been extensively documented in the literature.

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An Audit of the Consistency of Reporting Tumour Infiltrating Lymphocytes, and Ulceration in melanoma

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The Royal College of Pathologists Minimum Data Set in Malignant Melanoma includes tumour infiltrating lymphocytes (TILs) quantified in terms of brisk, non-brisk and absent, along with ulceration as important prognostic indicators. Various studies have also identified the anatomic location of the malignant melanoma as a prognostic variable. High risk areas include the back, upper arm, neck, and scalp forming the BANS concept.

A retrospective search of the hospital database for all cases of primary cutaneous melanoma between January 1999 and December 2003 was done. For each of the melanoma reports, the inclusion of TILs and the absence or presence of ulceration was recorded. The anatomic location of the melanoma was also noted.

The study included 204 patients over the 5 year period. Of the total number of reports, 22% were graded as brisk TILs, 29% non-brisk TILs, and 3% absent TILs. 7% recorded a perivascular lymphocytic response. 39% of reports gave no mention of TILs.

17% of reports recorded the presence of ulceration, 60% noted the absence of ulceration, and 22.5% did not mention.

Applying BANS:- 12.5% were on the back, 12% were on the upper arm, 2% on the neck and 0.5% from the scalp. These cases need follow up to monitor prognostic behaviour.

This audit highlights the importance of TILs and ulceration in melanoma.

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Melanoma With Myxoid Features - An Unusual Variant Of Primary Cutaneous Melanoma

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An 80 year old man presented with a six week history of a rapidly enlarging, painless nodule anterior to his left ear. The rapid growth of the tumour suggested the lesion was malignant, and the clinical differential diagnoses considered included squamous carcinoma, Merkel cell carcinoma, atypical fibroxanthoma and melanoma.

Histologically, there was a nodular tumour within the dermis composed of an admixture of spindle shaped cells and mildly pleomorphic, plump epithelioid cells. The cells were predominantly set within a hypocellular, myxoid stroma. The loose myxoid stroma and relatively bland spindle cell morphology was suggestive of a neural tumour. However, immunohistochemistry showed the tumour cells to be positive for S100, focally positive for HMB 45 and Melan A, and negative for cytokeratin. Focal melanin pigment was identified. The diagnosis of a malignant melanoma with myxoid features was made.

Until relatively recently, most of the reported cases of myxoid malignant melanoma described metastatic tumour deposits. There are only occasional published cases describing this pattern in primary cutaneous melanomas. This case highlights the importance of including melanoma in the differential diagnosis of cutaneous myxoid tumours and demonstrates the utility of employing additional immunohistochemical markers other than S100, especially when there is potential diagnostic confusion with neural tumours.

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Expression Of The Ki Ras 4A Isoform In Renal Cell Carcinoma

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We investigated the expression of the minor, 4A isoform of Ki Ras in renal cell carcinoma (RCC). Ki Ras is part of the growth signal transduction cascade in many cell types. It exists in two alternative splice isoforms, the ubiquitously expressed Ki Ras 4B and the alternative Ki Ras 4A which has a more restricted pattern of expression. Kidney is one of the small numbers of tissues in which the 4A isoform is expressed. Experimental data suggests that it may be important in the mineralocoricoid dependent growth receptor. We have examined the expression of the Ki Ras isoforms in a small series of RCC. RNA was extracted from snap frozen samples of RCC and subjected to reverse transcriptase PCR. We designed primer sets to be specific for the 4A isoform (forward - 5'tgtcatcttgcctccctac 3' Reverse 5'ttcctcagggctcaagagaa 3') and for common sequences in 4A – 4B (forward 5' ggcaaaattgtgcaagaggt 3' reverse 5' ttctcttgagccctgaggaa 3'). PCR products were run on 1.5% agarose gels and bands of appropriate size detected. The PCR product was directly sequenced and found to match the published sequence of Ki Ras 4A. These studies have confirmed that cells of RCC express the more restricted 4A isoform as well as the ubiquitous 4B isoform. We hypothesise that Ki Ras 4A has a growth promoting role, possibly mediating mineralocorticoid responses in renal cell carcinoma.

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DIAGNOSTIC QUANTITATION OF LYMPHOMA GENE SIGNATURES IN GLOBALLY AMPLIFIED POLYA cDNA

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Microarray gene expression profiling has identified "*Indicator*" genes predictive of outcome in diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL). Such methods use relatively large amounts of tissue and routine measurement of these genes is difficult. To test the use of *Indicator* genes as a diagnostic tool for lymphoma, we have used a simple, practical, polyA PCR based method for analysis of *Indicator* profiles in DLBCL and FL. PolyA RT-PCR was applied to RNA from 67 frozen human lymph nodes (LN). Resultant cDNA was analysed by real-time PCR for 36 *Indicator* genes; expression levels normalised to mean of four housekeeping genes. The *Indicator* genes distinguished between reactive and neoplastic LNs and between DLBCL and FL. ACTA expression was reduced in neoplastic compared to reactive LNs ($p<0.03$), whilst Urokinase ($p<0.00$) and KIA0233 ($p<0.01$) distinguished DLBCL and FL. Expression levels of EAR2, 5HT2B, HSF1, HSP27, ID2, KIA0233 & PKCG ($p<0.03$) were correlated with interval to death in FL, and BSP1 with interval to death in DLBCL ($p<0.04$). These results validate a simple, sensitive and robust method for *Indicator* gene analysis. Amplification using polyA PCR makes the technique applicable to very small clinical samples, including needle core biopsies, facilitating routine clinical application.

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Induction of apoptosis by leptomycin B in keratinocytes expressing HPV genes is mediated by high-risk E7 expression

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Non-genotoxic activation of p53 by leptomycin B (LMB) sensitises HPV-infected cervical carcinoma-derived cell lines to apoptosis but the mechanisms of this effect are unknown. In order to explore this phenomenon, we expressed HPV genes in primary human keratinocytes by retroviral transduction, and investigated the effects of LMB treatment in both monolayer and raft culture. Apoptosis was identified by immunodetection of both the M30 neoepitope of cytokeratin 18 and active caspase-3. The induction of apoptosis by LMB in monolayer culture was significantly greater in cells expressing HPV 16 E6/E7, and HPV 16 E7 than in control normal cells or cells transduced with the empty retroviral vector. Moreover, no significant induction of apoptosis was seen in cells expressing HPV 16 E6, HPV 6 E6 or HPV 6 E7 when compared with controls. When exposed to LMB in a pulse recovery experiment, cells expressing HPV 16 E7 and E6/E7 all died within 2 and 5 days of LMB removal respectively, whereas normal cells survived this treatment. Application of LMB to raft cultures of cells expressing HPV 16 E6/E7, the two HPV genes retained in high-grade cervical lesions, produced a dose-dependent effect. 50nM LMB induced widespread apoptosis and dissolution of the epithelium. These data indicate that LMB induces apoptosis in primary keratinocytes expressing high-risk, but not low-risk, HPV genes and that this effect is mediated predominantly by the E7 gene. However, a similar, although attenuated, effect was seen in cells expressing both the E6 and E7 genes. This indicates that LMB may prove useful as a topical therapy for HPV-associated anogenital disease. This is supported by preliminary evidence that LMB can also induce apoptosis in cells containing the whole viral genome.

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EXPRESSION PROFILING THE HUMAN SEPTIN GENE FAMILY AND IDENTIFICATION OF A NEW FAMILY MEMBER

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The septins are an evolutionarily conserved family of GTP-binding proteins involved in diverse processes including vesicle trafficking, apoptosis, remodelling of the cytoskeleton, infection, neurodegeneration and neoplasia. We report a comprehensive study of septin gene expression by DNA microarray methods in 10,360 samples of normal, diseased and tumour tissues. Validation of key probe sets has been performed by Q-RT-PCR. We have identified a novel septin, SEPT13 and show it to be related to SEPT7. We show that SEPT13 and the other known human septins are expressed in all tissue types but some show high expression in lymphoid (SEPT1, 6, 9 and 12) or brain tissues (SEPT2, 3, 4, 5, 7, 8 and 11). For a given septin some isoforms are highly expressed in brain and others are not. For example, SEPT8_v2 and v1, 1* and 3 are highly expressed in brain and cluster with SEPT2, 3, 4, 5, 7 and 11. However, a probe set specific for SEPT8_v1 with low brain expression, clusters away from this set. Similarly SEPT4 has lymphoid and non-lymphoid forms, SEPT2 has lymphoid and CNS forms and SEPT6 and SEPT9 are elevated in lymphoid tissues but both have forms that cluster away from the lymphoid forms. Perturbation of septin expression was widespread in disease and tumours of the various tissues examined, particularly for conditions of the CNS where alterations in all 13 septin genes were identified. This analysis provides a comprehensive catalogue of the septin family in health and disease. It is a key step in understanding the role of septins in physiological and pathological states and provides insight into the complexity of septin biology.

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Chronic Renal Graft Dysfunction: A Role for CD103+ T Cells in Epithelial to Mesenchymal Transition?

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A recent protocol biopsy study has confirmed that acute rejection is a risk factor for chronic allograft nephropathy, which is mainly caused by the loss of renal tubules with concurrent interstitial fibrosis. In mouse models this pathology is produced by TGFβ which induces phosphorylation of Smad 2/3 leading to phenotypic transition of tubular epithelial cells (TEC) to activated fibroblasts, known as epithelial to mesenchymal transition (EMT). A sub-population of infiltrating intraepithelial T cells (IEL) in transplant biopsies has previously been shown to express CD103, now known to be a marker of TGFβ-producing regulatory T cells. In this study we examined the potential of IEL to induce human TEC to undergo EMT after renal transplantation.

Immunohistochemical investigation of transplant biopsy specimens showed that during acute rejection upregulation of the early EMT marker, S100A4 (fibroblast specific protein) was associated with nuclear translocation of pSmad 2/3; both of these markers colocalised with infiltrating intraepithelial T cells.

Cultured primary human TEC also upregulated S100A4 and pSmad 2/3 expression following co-culture with allo-activated T cells, which had been induced to express CD103 by stimulation with TGFβ.

Intratubular allospecific CD103+ve T cells have the potential to induce EMT following renal transplantation, leading to chronic graft failure.

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Mortalin Is Overexpressed By Colorectal And Breast Carcinomas

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Mortalin, also known as mitochondrial heat-shock protein 70 (mhsp 70), is involved in cell cycle regulation with important roles in cellular senescence and immortalisation pathways. It is known to bind and inactivate wild-type tumour suppressor protein p53 and modulates the Ras-Raf-MAPK pathway.

Using comparative proteomic analysis we identified over-expression of mortalin in colorectal adenocarcinomas. Immunostaining a tissue microarray from a large well-defined series of colorectal cancers enabled us to determine the clinicopathological significance of the proteomic and immunocytochemical findings. We found that mortalin over-expression by colorectal cancers correlates with poor patient survival independently of standard prognostic variables.

The importance of this protein in the neoplastic process is further suggested by our findings that colorectal adenomas and other microarrayed cancers strongly over-express mortalin. In the breast, both ductal carcinoma-in-situ and invasive ductal carcinomas show strong mortalin staining compared to benign breast tissue.

Our findings suggest that mortalin is fundamentally involved in colorectal and breast neoplasia and demonstrate that over-expression may predict poor clinical outcome in patients with colorectal cancer.

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Geminin is a critical regulator of growth in human somatic and germ cells

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The origin licensing repressor geminin provides an intriguing molecular link between the cellular circuits controlling proliferation, differentiation and genomic stability. To understand how this unique bi-functional molecule might co-ordinate proliferation and differentiation in human cells, we studied geminin's function and biochemical properties in a cell-free replication assay, and resolved its structure by transmission EM of negatively stained protein samples and single-particle analysis. Our data provide the first molecular structure of full-length geminin, and show that the molecule is a tetramer formed by two dimers with monomers interacting via coiled-coil domains. Functional studies of geminin in a human *in vitro* replication assay show that establishment and maintenance of G0 is independent of geminin, and that down-regulation of geminin during the G0-S transition is critical for escape from quiescence. Linkage of geminin's molecular blueprint and our functional data to expression profiling in human tissues has provided us with new insights into the molecular mechanisms by which geminin coordinates proliferation, differentiation and genomic stability. Our studies show that replication initiation is regulated by very different mechanisms in somatic and germ cells, and have revealed a striking sexual dimorphism in the mechanisms repressing origin licensing and preventing untimely DNA synthesis during meiosis I, implicating a pivotal role for geminin in maintaining integrity of the male germ line genome.

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The Role Of The Oncogene p28^{GANK} And Chronic Hepatitis C (HCV) In The Development Of Hepatocellular Carcinoma (HCC)

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Introduction: HCC is one of the most prevalent cancers globally. Chronic inflammation has a suspected role in hepatocarcinogenesis. HCV may have a role in hepatocarcinogenesis. A novel protein, p28^{GANK}, was recently identified in HCC and proposed as a potential carcinogen. Aim: Studying the role of p28^{GANK} in hepatocarcinogenesis and determining its relationship with the chronic inflammatory process mainly by HCV. Methods: Thirty HCC (9 were HCV positive) specimens, were studied for p28^{GANK} expression, with 40 foetal, normal, fatty and cirrhotic liver specimens. Immunohistochemistry was done for p28^{GANK} using a specific polyclonal antibody. Fifteen out of 30 HCC specimens with twelve negative control specimens were selected for analysis of p28^{GANK} mRNA (4/15 were HCV positive) by RT-QPCR. We used IHC for detecting the HCV proteins by using specific monoclonal antibodies. Studies of the chronic inflammatory process were conducted using IHC of cyclo-oxygenase 2. Results: p28^{GANK} expression was absent in the normal, fatty and cirrhotic livers. Eighteen out of the 30 cases of HCC showed p28^{GANK} positivity, mainly nuclear. Only four out of these 18 cases showed p28^{GANK} expression in carcinoma area (1/4 was HCV positive) while 17/18 showed strong preferential expression in para-carcinoma tissue (6/17 were HCV positive). In contrast, 9/15 of the cases studied by the RT-QPCR, showed higher mRNA signals of gankyrin in the carcinoma area. No robust anti-HCV antibody for IHC was found. For the COX-2 immunostaining, 9/30 HCC cases showed positive cytoplasmic expression in the carcinoma and none in the para-carcinoma areas. Seven of these 9 cases were positive for gankyrin. Conclusion: Our findings are consistent with a role for p28^{GANK} at an early stage of hepatocarcinogenesis.

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Tenascin-C isoform expression in colorectal cancer

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The ECM protein TN-C is up-regulated in many solid tumours and we have previously identified tumour-specific isoforms in breast carcinomas. This study aimed to analyse TN isoform profile in colorectal tissues and to relate this to invasive capacity.
Normal colon (n=26), adenoma (n=18), colorectal cancer (n=29) and inflammatory bowel lesions (n=8) were analysed by immunohistochemistry using antibodies recognising total TN (BC24) and exon-14 containing TN isoforms (alpha IIIB). RT-PCR analysis was carried out using primers spanning the alternatively spliced region of TN. The invasive capacity of 5 colorectal cell lines was measured and correlated with isoform profile.
In normal colon and adenomas, TN was present in basement membrane with larger, exon-14 containing isoforms in muscularis mucosae and muscularis propria. In both invasive carcinomas and inflamed lesions, larger TN isoforms were identified in the stroma. RTPCR identified multiple TN isoforms in all sample types, with no clear differences in the pattern of expression. The tumour-associated AD1 exon was detected in normal and tumour colon. Truncated and higher MW isoforms were identified in 3 cell lines (SW480, SW620, HT29), whilst 2 cell lines did not express TN (HCT116, HT15). There was no clear correlation between TN isoform expression and invasive capacity.
In conclusion, this study has demonstrated multiple TN isoforms in normal colon and, in contrast to breast there are no consistent changes in isoform profile with tumour progression. Furthermore, it has established that AD1 is a normal component of colon, probably smooth muscle, and this is likely to reflect a highly specific structure-function relationship, although a direct relationship with invasion is not evident.

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Cell proliferation in oesophageal carcinomas is associated with the expression of cyclin A but not with that of cyclin B, D1, or E.

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Cyclins are key regulators of cell proliferation, operating to permit progression through the cell cycle via association with specific members of the cyclin dependent kinase (CDK) family. High levels of individual cyclins in tumours may be interpreted as either specific molecular disturbances which may lead to inappropriate cell cycle progression, or simply as a reflection of a high proliferative fraction. To investigate the expression patterns of the cyclins in oesophageal carcinoma, 80 oesophageal cancers (18 squamous carcinomas, 62 adenocarcinomas) were arrayed in triplicate in tissue microarrays, and immunohistochemistry (IHC) was performed for cyclin A, cyclin B, cyclin D1 and cyclin E. Ki-67 IHC was also performed. Staining was assessed as the proportion of tumour nuclei with positive staining, and scores from triplicate cores combined. Cyclin D1 expression was significantly lower in adenocarcinomas than in squamous cell carcinomas ($p<0.001$), consistent with cell division being initiated by cyclin D1 independent pathways in at least some adenocarcinomas. There was no statistically significant correlation between the expression of any of the 4 cyclin molecules and tumour size, tumour grade, lymph node metastasis status or vascular invasion, for either squamous carcinomas or adenocarcinomas. In multivariate analysis, cyclin A was independently associated with tumour cell proliferation for both squamous carcinomas and adenocarcinomas ($p<0.001$). In normal oesophageal epithelium, cyclin A expression was restricted to the suprabasal layer. The observed association of cell proliferation with cyclin A levels, independent of the levels of other cyclins, is consistent with cyclin A over-expression being a specific molecular disturbance in this context.

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An Audit of the Diagnostic Value of Fine Needle Aspiration Cytology in Thyroid Disease

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Background: Thyroid fine needle aspiration cytology (FNAC) is simple and cost effective and is more sensitive than other modalities in the detection of malignancy. Although mainly a triage procedure, it may be diagnostic for certain lesions allowing treatment planning by selecting nodules requiring surgery, thus reducing the number of thyroidectomies.
Objective: To audit the accuracy and value of FNAC in thyroid nodule assessment over a 4 year period.
Setting: Histopathology department at district hospital.
Patients: Computerized records from cytological and histological examinations of 187 thyroid specimens (168 women,19 men) from 2001 to 2004 were studied.
Outcome measures: Histology and cytology were correlated.
Results: 54 aspirates (29%) were unsatisfactory and 98 (52%) were benign. 33 (18%) were atypical. 8 (4%) showed features suspicious of malignancy. 2 (1%) were malignant. Histology was available for 44 cases. Statistical analysis yielded the following: sensitivity 50%, specificity 98%, positive predictive value 71%, negative predictive value 96% and accuracy 94%.
Conclusion: Highly accurate with a relatively low rate of false negative and false positive diagnoses, problems are mainly due to interpretative difficulties with malignant diagnoses based on minimal atypical cytologic features or overlapping cytologic features of follicular neoplasms with the follicular variant of papillary carcinoma.

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Compliance With The Minimum Dataset For Reporting Endometrial Cancer

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Aim: Endometrial cancer is the fourth most common cancer in women. Our aim was to assess compliance with the Royal College of Pathologists minimum dataset, and to assess what and how information was being recorded, and from the information gained develop an electronic proforma to improve compliance.
Methods: 90 consecutive cases, 45 from the Leicester Royal Infirmary and 45 from the Leicester General Hospital between 2001 and 2004 were assessed. The cases were anonymised and the data recorded in the reports was compared against the national guidelines.
Results: Of the 17 components of the minimum dataset, we were 100% compliant in only 3. Of the remaining components we were up to 37% non-compliant in recording microscopic myometrial invasion and 26% non-compliant in recording involvement of the uterine serosa. A number of problems were recognised including not recording negatives and multiple methods of assessing and recording data.
Outcome: Compliance with the minimum dataset was poor. From the information obtained an electronic proforma was written incorporating the full grading and staging systems. The initial results of using an electronic proforma are promising. Full compliance and agreement on best methods of assessing and recording vital prognostic information can be achieved using electronic datasets.

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Good Practice In Communication About Postmortem Examinations

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The Retained Organs Commission identified a need for communication skills training for pathologists, and a request for training was made to the local Regional Council of the Royal College of Pathologists. The Medical Education Unit of our employing University runs workshops on *Advanced Communication for Clinicians: Breaking Bad News*, and the programme was adapted, using simulated patients and fictional autopsy reports. The programme was offered to doctors within all laboratory disciplines in the NHS Region.

Two workshops have occurred, with 17 participants. Trainees predominate, with only two consultants; all were histopathologists.

	1	2	3	4	5	6
How confident in communicating with relatives did you feel *before* this workshop? 1 = not at all; 6 = very	1	2	8	3	3	
How confident in communicating with relatives did you feel *after* this workshop? 1 = not at all; 6 = very					10	7

The feedback from these participants indicates that the principal aim, that of giving pathologists confidence to talk to the bereaved, was achieved.

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Thyroid Fine Needle Aspiration Cytology : Adequacy and Accuracy

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Fine needle aspiration cytology (FNA) of thyroid nodules is a minimally invasive method of distinguishing between benign and malignant thyroid nodules. The purpose of this study was two fold, 1) to evaluate the FNA adequacy rate and 2) to assess FNA diagnostic accuracy by correlating the FNA diagnosis with the histological diagnosis following surgical resection. 648 FNA's from 447 patients received by the Pathology Departments of the South Glasgow NHS Trust between 2000 and 2004 were included. The overall inadequacy rate was 39%. In 132 FNA's from 102 patients, FNA and histological diagnosis could be compared. The sensitivity of FNA (66%), specificity (75 %) and other test parameters are presented. In this retrospective study we were unable to further separate inadequacy rates of FNA's e.g. by operator or sampling method. This highlights the need for a prospective study.

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Appraisement: Tinkering At The Edges Of Appraisal And Assessment

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Aim: To design a single transferable electronic portable appraisal and assessment training (e-) portfolio for Specialist Registrars in Histopathology.
Materials and Methods: Histopathology Specialist Registrars (SpR) are appointed to their posts that rotate between one or more recognised Royal College of Pathologist training institutions, each of which have an Educational Supervisor and several Consultant Trainers. Each SpR is expected to have six-monthly appraisals (n=9) and annual RITA's (n=5) over their 4½ year training period.
Results: We have designed an e-portfolio that we have used in our institution for the past four years and which can potentially be transferred between training institutions. It contains all information relating to each SpR (including the NHS Training Portfolio). This e-portfolio tracks SpR progress through their training cycle, addresses almost every aspect of their training requirements, allows for assessment against standards, reveals training deficiencies between each appraisal cycle, allows for corrective measures to be instituted, informs each RITA and also allows for assessment of "competencies" in diagnostic pathology. The document is robust and, being electronic, can easily be adjusted to the deficiencies in the document itself.
Conclusions: We have designed a portable, transferable appraissment (appraisal and assessment) e-portfolio for SpR's in Histopathology that tracks their training progress, addresses their development needs, informs their RITA and, can be used to address "competencies" and "unsupervised" histopathology reporting. It can be modified to be central, secure and web-based.

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Designing Guidelines For Mortality Review.

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Aim: To define and introduce guidelines for a Mortality Review (MR) process within a (Trust) health-care setting.
Material and Methods: MR was identified as an important Clinical Governance issue by our Trust and, in 2000 the post of Chairman was formalised and the MR Committee (MRC) was established.
Results: During the course of the next 18 months we established the membership of the MRC, developed guidelines for MR within the community of the Trust and designed a MR Process (MRP). Our challenges were those of inclusivity, threat of litigation, discoverability of information, funding constraints and establishing the need for professional time resource. The latter two continue to be a brake on full compliance with the Guidelines that we established and ability to review all, rather than a percentage, of all deaths. We were able to secure CPD points, to an annual maximum of 12 for Consultants involved in the MRP.
Conclusions: MR is a Clinical Governance and risk management process that is essential tool for monitoring, evaluating and improving standards in patient care and management. It provides a forum where information can be collated, trends can be identified, Directorate and cross-Directorate MR issues can be discussed and eventually disseminated within and between Directorates and, where necessary, to Trust management. MR also identifies cases that can be used as teaching and learning resource for all categories of staff, trainees and students. It is especially important that Trust management is committed, supports and appropriately funds this process.

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Talk To Me! Is There Appropriate Laboratory Communication Between Pathologists And Biomedical Scientists?

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Aim: To determine whether Biomedical Scientists (BMS's) or Pathologists, or both, are aware of the meaning of requests for "further " H/E slides from a paraffin block.
Materials and Methods: Pathologists (n=4) and BMS's (n=9) from the Royal Cornwall Hospital (RCH) and University College Hospital (UCH), Nigeria (n=3 and n=2 respectively) were given questionnaires designed to evaluate their understanding of the depth in microns associated with routine laboratory requests for 1) levels, 2) shallow steps, 3) thick steps, 4) deeper, 5) deeper to cover and 6) serial sections.
Results: None of the RCH pathologists or BMS's were consistent in their understanding of the depth for levels (range12-20µm vs 12-40µm), shallow steps (range 8-16µm vs 4-24µm), thick steps (range 28-40µm vs 20-80µm), deeper (range 24-40µm vs 20->40µm), or deeper to cover (40µm vs 20->40µm). In contrast, UCH pathologists and BMS's returned values for levels (range 8-12µm vs 12-16µm), shallow steps (range 4µm vs 4-12µm), thick steps (range 12-16µm vs 10-16µm), deeper (range 12-20µm vs 16-20µm), deeper to cover (16µm vs 10-16µm) and serial sections (range 1-5µm vs 4-10µm).
Conclusion: This study demonstrates that there is inconsistency, and lack of understanding, among and between pathologists and BMS's with respect to the depth at which extra sections should be cut in each of six common categories of laboratory requests for "extra sections". Clearly there is a need to establish uniformity of communication between pathologists and BMS's within, and probably also between, laboratories.

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Audit Of "Suspicious''(C4) And "Malignant''(C5) Categories Of Fine Needle Aspiration of Breast

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Fine needle aspiration cytology (FNAC) and core biopsy form a vital part of assessment of breast lesions. The rationale of this audit was to check on the predictive value of the C4 and C5 categories. Thus our aim was to
1. Audit the diagnostic performance of FNAC for C4 and C5 categories with histopathology correlation.
2.To analyse and establish positive predictive value (PPV) of C4 and C5 category against the local set standard.
This was a retrospective analysis of reports of all cases, between 1/10/2003 and 30/09/2004, labelled C4 and C5 cytologically which were compared to the final histological diagnosis.
The total number of FNAC's was 557 of which C4 and C5 were 95. Of 56 C5 lesions, 11 had no histology correlation as patients elected not to undergo any further investigations or surgery (too elderly/frail). The remaining 45 lesions were all malignant. Of 39 C4 lesions, 2 had no histology, 3 cases were benign (2 fibroadenomas and 1 organising haematoma) whilst 34 were confirmed as malignant.
In conclusion
1. The PPV for C5 lesions is 100% and C4 is 91.9%
2. Both these values match the local set standard.
3. Re-audit.

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Multidisciplinary Team Meetings- Are They The Best Use Of A Pathologist's Time. Audit Of Practice In A District General Setting.

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Over the past decade, multidisciplinary meetings have allowed for better communication between pathologists and other clinicians. The time spent preparing for and attending these meetings, means there is erosion of time available for reporting specimens and CPD. Over a 4 weeks period, we asked pathologists and secretarial staff in our department to fill in a detailed questionnaire about each MDT. 17 meetings were attended. On average, 12 cases (range 5-34) were reviewed in each meeting. Secretarial staff spent an average of 100 minutes (range 30 –270) preparing each meeting. Pathologists took 76 minutes (range 30-270) to prepare and an average of 69mins(range 45-120) attending each meeting. 203 cases (20% of biopsy caseload) were reviewed. In 19 cases (9.3%), the pathologist felt their presence at the meeting had clarified the original report leading to a more informed decision regarding management. 6 cases had a supplementary report issued following discussion with the reporting pathologist. No serious diagnostic errors were identified . There were minor inaccuracies only. A move towards virtual MDT s for most cases may be beneficial. In the 10 % of more complex cases, the team could continue to meet in person with less impact on service provision.

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Histopathology in the foundation two (F2) year – Will it work?

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Background: 'Modernising medical careers' aims to reform post-graduate education establishing a broader base of specialist training, providing excellent opportunity to recruit and retain trainees. The Royal College believes that F2 attachments in histopathology would be valuable. There is little evaluation of the F2 pilots in histopathology.
Aims The questionnaire is designed to assess final year students' knowledge and exposure to histopathology. Questions are aimed at evaluating the understanding of practical aspects of histopathology, difficulties surrounding diagnosis, the scientific basis and attitudes towards histopathology in the F2 year or as a career. We aim to devise and correctly pitch F2 attachments locally.
Methods With permission the questionnaire is distributed to the students simultaneously in year five.
Results The questionnaire has been piloted showing that students had little exposure and understanding of the practical aspects of histopathology, 9% still believed that we see patients. Encouragingly, attitudes changed after a pathology attachment, students recognised histopathologists spend more time on microscopy, 31% rather than 24% before. Students were more interested in a career in histopathology, 36% rather than 6%, and were less deterred by the scientific basis, 9% rather than 27%.
Conclusion. F2 attachments may improve understanding of histopathology as well as assisting recruitment.

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Recommendations for Cervical Cancer pathology reporting: A local audit to compare standards with minimum datasets

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Aims: Accurate pathological staging of cervical cancer provides important prognostic information. The aim of this study was to compare the standard of pathology reporting of cervical cancer resections at a large teaching hospital in the UK with standards set by the Royal College of Pathologists.
Methods: All reports for cervical cancer resections from the teaching hospital since March 2001 were collected. Individual items of information were compared with minimum datasets provided by the Royal College of Pathologists. Items were classified as "mentioned", "not mentioned ", or "not required".
Results: Thirty- two reports were audited. Surprisingly 60% of the reports had at least one data item missing. Gross description of the macroscopic dimensions of the uterus was the most commonly omitted data item. Presence or absence of CIN, CGIN and their grade was not indicated in several reports. A significant proportion of the reports had more than one data item missing.
Conclusion: To ensure adequate compliance it is recommended that a proforma be attached to the referral forms at cut up. Introduction of an electronic proforma that can be sent along with the reports will have a huge impact on reauditing. This would however require updating of the IT systems.

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An audit into the appropriate use of cytopathology in the evaluation of cerebrospinal fluid in a teaching hospital

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Aim: To ascertain the number of CSF specimens that fulfilled the clinical criteria for CSF examination, as suggested by Mackenzie and colleagues, 1996.
Method: Clinical and pathological data from every CSF submitted for cytological evaluation in 2004 were reviewed. The following criterion was used to decide if cytological examination of CSF was required: clinically known or suspected CNS malignancy. The clinical details on each request form were recorded and whether the patient had microbiological investigation of the CSF or appropriate radiological imaging.
Results: 90 CSF specimens were evaluated. Radiological imaging in 3% (3) of patients was diagnostic of a central nervous system neoplasm. Clinical details were sufficient to warrant cytological evaluation in 12 (13%) cases. 3% (3) of CSF specimens were diagnostic of malignancy and 97% were not diagnostic. 95% of the specimens were also sent for microbiological investigation.
Conclusion: Of the 90 CSF specimens sent for cytopathological examination, only 13% conformed to the clinical criteria set out by Mackenzie and colleagues.
Guidelines are not easily available to clinicians to ascertain whether cytological examination of CSF is indicated; it is apparent from our study that if a lumbar puncture is performed there is a tendency for clinicians to send the fluid for microbiological and cytopathological analysis. We hope to provide accessible clinical guidelines for clinicians in order to reduce the number of unnecessary investigations.

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How Helpful Is Cytokeratin 19 Immunohistochemistry In Diagnosing Papillary Thyroid Carcinoma?

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Introduction and aim: Papillary Thyroid Carcinoma (PTC) is the most common malignant neoplasm of the thyroid but histological diagnosis can be problematic because the characteristic nuclear features may be subtle. The aim was to assess the value of CK19 immunolabelling in distinguishing PTC from non-PTC lesions.

Methods: CK19 immunohistochemistry was performed on 84 lesions including 59 PTCs (42 definite and 17 possible on H&E), follicular neoplasms, hyperplastic nodules and lymphocytic thyroiditis. Intensity, cellular localisation and distribution of immunostaining were assessed in the lesions and surrounding thyroid.

Results: All PTCs were CK19 positive, as were many non-PTC lesions, but the staining patterns were different. Epithelial cells of PTCs showed moderate to strong staining of cytoplasm and membranes throughout. Non-PTC lesions showed weak patchy staining sometimes with membranous localisation. Significant variations from this trend occurred with lymphocytic inflammation, in Hurthle cells and multinodular goitres.

Conclusion: CK19 immunopositivity has high sensitivity but low specificity for PTC. The variation in staining patterns assists in differentiating PTCs from other thyroid lesions. Knowledge of CK19 staining characteristics is essential for correct interpretation.

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Perivascular and Intravascular Invasion in Follicular Thyroid Carcinoma

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For surgical pathologists estimation of vascular invasion is a routine part of the assessment of thyroid carcinomas, However the relationship between vascular invasion and prognosis in follicular thyroid carcinoma is debated. Vascular invasion is less common in malignant melanoma but has been re-evaluated by Lugassy and Barnhill and his colleagues in recent studies. These have suggested that, in some cases, melanoma infiltrates and spreads around the wall of blood vessels rather than within the true vascular space (extravascular migratory metastasis).
We used immunohistochemistry to study the pattern of vascular invasion in all of our cases of follicular carcinoma of the thyroid in which vascular invasion had been reported. More than 30 individual vessels were assessed by CD31,CD34 and Factor VIII staining. Although histological assessment was somewhat subjective we recorded intravascular tumour in 42% of vessels, perivascular tumour in 50% of vessels and what could be both perivascular and intravascular tumour in 8% of vessels. No one antibody was consistently superior and the observations were made from a combination of the three preparations.
Our results indicate that thyroid carcinoma invades both around and within blood vessels. An obvious inference is that perivascular invasion is merely the prelude to vascular wall invasion and haematogenous spread. As the relationship between vascular invasion and prognosis is uncertain we suggest that surgical pathologists should carefully evaluate the pattern of tumour spread around thyroid blood vessels and report it in precise terms. This should be evaluated prognostically.

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Inadequate FNAC of the thyroid: A review of 268 patients and correlation with histological or cytological evaluation

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Background and Aims: The fine needle aspiration cytology (FNAC) of the thyroid is the predominant method of pre-operative investigation of thyroid lesions. The routine use of fine needle aspiration has reduced the rate of unnecessary surgery for thyroid nodules. However, there are overlaps in the criteria for defining adequacy in thyroid aspirates. A proposal for avoidance of non-diagnostic or inadequate aspirates is presented devised using clearly defined criteria which includes the presence of six or more groups of follicular cells and each having more than ten readable, viable cells. Our aims were to determine the reasons for the high percentage of inadequate thyroid smears and to apply standard criteria for defining the adequacy of smears and to correlate results with histological and cytological outcome.
Methods: The results of 268 patients with inadequate thyroid indexed smears are presented over a period of fifteen years extending from 1986 to 2000. For statistical analysis, only those patients with subsequent histological or cytological analysis were included. Slides were reviewed by two observers.
Results: Out of 268, 71 (26.5%) met with the standard criteria and were found to be adequate smears on review. Of these 71, 5 (9.9%) showed malignancy, 11 (15.5%) showed adenoma and 50 (74.6%) showed benign hyperplasia. In addition 11 (4.1%) cases were classified as cystic degeneration/ cysts. The slide preparation error was noticed in 26 (9.7%) and the aspirator's fault was observed in 160 (59.7%) cases. **Conclusion:** The use of well-defined criteria for adequacy is helpful because it improves the diagnostic efficiency of thyroid FNA. It also avoids unnecessary surgery for benign non-neoplastic thyroid lesions and expedites the surgical requirement for neoplastic lesions.

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Mutation And Tissue Microarray Analysis Of Tumour Suppressor Genes And Cell Cycle-Related Proteins In Colorectal Cancer

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We have characterised a series of 107 sporadic colorectal cancers for mutations in p53, K-ras and APC genes and related the findings to patient outcome. Patient survival was analysed using Log Rank and Cox proportional hazard statistics. Only K-ras mutations were found to be associated with poor patient prognosis (p=0.010). This observation was confirmed by multivariate analysis correcting for Dukes' stage, age and sex [Hazard Ratio 2.9, p=0.0040 (1.4-6.2; 95% confidence interval)].

In addition, immuno-histochemical analysis of p53, p21, p27, Mdm2, Bcl2 and ß-catenin proteins was carried out on a tissue microarray containing tumour and normal cores from 98 of the original tumour series. Analysis of the tumour immunoprofiles showed that over-expression of p53 protein positively correlated with the presence of p53 mutation (Fisher's exact test p=0.005) and p27 expression negatively correlated with K-ras mutation (Fisher's exact test p=0.013). Of the proteins studied, only the presence of ß -catenin correlated with patient survival; expression of nuclear ß-catenin was associated with better patient prognosis (p=0.023), where multivariate analysis correcting for Dukes' stage, age and sex gave a Hazard Ratio of 0.379, p=0.003 (0.199-0.722; 95% confidence interval)].

These results form part of a unique dataset on a single patient cohort. We hypothesise that K-ras mutation and nuclear ß-catenin expression may be important prognostic variables requiring further evaluation.

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DNA Ploidy, S Phase Fraction, P53 Expression And Chromosome # 17 Copy Number Aneusomy In Colorectal Carcinoma In Egypt.

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BACKGROUND: DNA ploidy, S-phase fraction (SPF), p53 Immunohistochemistry (IH) and chromosome # 17 aneusomy studied in a series of 30 patients < 35 years old undergoing resective surgery for primary operable colorectal cancer (CRC) to investigate whether these changes have any clinical value in predicting CRC patient's prognosis.
METHODS: DNA ploidy & SPF performed on tumour frozen tissues by flow cytometry (FMC), chromosome copy number changes using non-radioactive fluorescent Insitu hybridization (FISH) using centromer specific DNA probe (D17Z1) for chromosome # 17 and p53 overexpression by IH on paraffin embedded tissues. The median follow up time was 36 months.
RESULTS: DNA ploidy was associated with high Histologic grade (G3) ($p<0.05$), high SPF (>15.6) in 43 %, lymph node metastases ($P<0.01$), advanced Duke's stage (C&D) ($P<0.01$) and distal tumours ($P<0.001$). FISH showed chromosome #17 aneusomy in 26.6 %, (20 % deletion and 6% gain) while p53 overexpression by IH in 59.4 %. No association between chromosome #17 aneusomy by FISH and p53 expression by IH.
CONCLUSIONS: DNA aneuploidy, high SPF, p53 overexpression are the hallmark of CRC in young Egyptian patients and are predictors of poor outcome.

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Fascin expression is increased in a size-dependent manner in colorectal adenomas and promotes cellular motility.

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Dynamic rearrangements of the actin cytoskeleton are necessary for a range of cellular processes including cell motility. Aberrant motility and subsequent tissue invasion are key hallmarks of progression of colorectal adenomas to invasive carcinomas.
Fascin is an actin bundling protein overexpressed in several cancers. We have previously shown that Fascin expression is not detected in normal colon epithelial cells but is found in a subset of colonic carcinoma.
In this study we examined the expression of Fascin in the early stages of the adenoma-carcinoma sequence in the colon. Immunohistochemical analysis of 64 colonic adenomas showed that Fascin expression correlated with tumour size. Six out of 14 small adenomas (<0.5cm), 23 out of 30 medium sized adenomas (<0.5cm and <3.5 cm) and 18/20 large adenomas (>3.5cm) showed fascin immunoreactivity. Furthermore, epithelial Fascin expression in a proportion (17/20) of large adenomas tended to be focused more often around the stalk of the polyp compared to small (2/14) and medium sized adenomas (15/30) suggesting a possible correlation with invasive potential. To assess the functional consequences of Fascin overexpression in colorectal tumour progression, migration assays were carried out on both colonic adenoma and carcinoma- derived cell lines forcibly overexpressing Fascin. Cell motility was significantly increased in the Fascin transfectants compared to controls. These data suggest that fascin expression is upregulated during colorectal tumourigenesis and this may play a role in the progression towards a malignant phenotype.

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Lymph Node Sampling in Oesophagogastric Cancer – A Literature Review and An Audit Study

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In many cancers the number of lymph nodes required for accurate N staging is uncertain. Techniques have been suggested for increasing lymph node yield. The Royal College of Pathologists recommends that a minimum of 15 lymph nodes be sampled to accurately stage an oesophageal or gastric cancer. Recent studies, however, suggest that as many nodes as can be sampled are required in the case of oesophageal cancer and that 10 nodes are adequate to stage an N0 gastric cancer, but 20 or 30 nodes are needed for stages N1 – N3.
In this audit the adequacy of lymph node sampling against an agreed standard of 15 nodes is assessed and the effect of separate submission of lymph nodes on node yield evaluated.
In total 96 specimens were received over the period of study. An average of 21.8 lymph nodes where harvested per specimen and 76% of specimens had 15 or more nodes harvested. Separate submission of nodes significantly increased the yield of nodes (23.2 vs 17.5, $p<0.05$).
In conclusion, the number of lymph nodes required to accurately stage an upper GI cancer remains uncertain, however, surgeons should be encouraged to identify and separately submit lymph nodes as this increases node yield.

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Accuracy and Adequacy of Upper GI Cancer Pathology Reporting – An Audit Study

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There has been a move towards a multidisciplinary team approach (MDT) to the management of upper GI malignancy. Validation of this process requires audit.
The role of the pathologist is providing an accurate pTNM stage and other relevant prognostic information in his/her report. The Royal College of Pathologists produced minimum datasets for reporting upper GI cancers, these have subsequently been modified by various groups. This audit assesses the impact of subspecialisation and introduction of a synoptic report for upper GI cancers on the adequacy of pathology reports compared to an audit pre-subspecialisation.
Pre-subspecialisation 10 pathologists reported resection specimens and reports contained an average of approximately 70% of required dataset items. This compared to post-subspecialisation and introduction of a synoptic report when 2 pathologists only reported the specimens and all reports included 100% of dataset items.
In conclusion, this audit demonstrates that subspecialisation of pathology reporting, the introduction of a standard computer generated synoptic report for upper GI cancers and an MDT approach have significantly improved the completeness and accuracy of pathology reports.

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Morphological Changes After Palliative Stenting for Upper GI Cancer – A Report of Two Cases

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In cases of upper GI malignancy where curative resection is not possible, a palliative stenting procedure may be attempted to relieve symptoms of obstruction. Complications may lead to the stent being resected or stents may be removed at autopsy. Stent-induced tissue changes have not, to our knowledge, been described in the literature.
The morphological changes present in two cases where stents were resected due to complications are described.
In case 1, a pyloric stent was resected from an 83 year old female with previous gastric lymphoma. The mucosa showed an unusual, regularly spaced pattern in the glands in addition to ulceration, fibrosis, and regeneration.
In case 2, an oesophageal stent was resected from a 45 year old male. In addition to ulceration, fibrosis and scarring, a mould inprint of the stent was identified in the squamous epithelium.
These findings highlight a spectrum of expected and unexpected stent-induced morphological changes, the significance of which needs to be explained. An understanding of these changes may provide an explanation for the complications of these stents.

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Xanthogranulomatous Appendicitis - An Unusual Healing Pattern Of Appendicitis

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Introduction
Xanthogranulomatous inflammation (XGI) is an uncommon type of chronic inflammation that has been described in various organs. However, there are very few reports in the literature describing this entity in the appendix. We report a case of xanthogranulomatous appendicitis.
Case report
A 50 year old man was admitted as an emergency with a history of lower abdominal pain, diarrhoea and abdominal distension. CT scan of the abdomen showed a 5 cm inflammatory mass near the appendix. Acute symptoms and signs settled on treatment with antibiotics. Four months later an interval appendectomy (IA) was performed. On gross examination the appendix was fibrotic at the tip but not obviously inflamed. Microscopically large aggregates of foamy macrophages were seen in the mucosa.
Discussion
XGI is a secondary phenomenon of chronic suppuration. Other putative pathogenetic mechanisms include defective lipid transport, immunologic disturbance, hypoxia, infections due to low-virulence organisms, reactions to specific infectious agents and lymphatic obstruction. Histologically it is characterised by a prominent component of foamy macrophages. Our case occurred in an IA specimen. A recent study reports that XGI occurs in IA but not in acute appendicitis specimens. In summary, xanthogranulomatous appendicitis is an unusual healing pattern of appendicitis. Its pathogenesis is probably multi-factorial.

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P53 Expression in Mucinous Gastric and Colonic Adenocarcinoma

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Introduction: The prognosis of mucinous carcinoma of the gastrointestinal tract is controversial. Generally they are associated with poor prognosis when compared to non-mucinous tumours.
Aim: We investigated by immunohistochemistry the over expression of P53 in gastric and colonic mucinous carcinomas for comparison and correlation to prognosis.
Materials and Methods: Twenty cases of colonic mucinous carcinoma and ten gastric mucinous carcinoma were selected excluding signet ring cell carcinoma. Formalin-fixed paraffin-embedded tissue sections were immuno stained with monoclonal anti P53 Dako clone D07. The level of P53 expression was scored semiquantitively based on staining intensity less than 5% staining was considered negative. Nineteen colonic carcinoma (95%) expressed P53 with a range of 20 – 90% positivity. Only six gastric carcinoma (60%) expressed P53 with 15 – 40% range.
Conclusion: Despite the fact that gastric mucinous carcinoma carry a worse prognosis than colonic mucinous carcinoma, the P53 expression was demonstrated in only 60% of cases, in addition they exhibited a lower percentage of cell expression than colonic carcinoma.

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Radiological and pathological response rate to pre-operative radiotherapy in 32 patients with locally advanced rectal carcinoma

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Purpose: To assess quantitatively the degree and rate of tumour regression following preoperative radiotherapy in locally advanced rectal cancer.
Materials and Methods: Thirty two patients who completed long course preoperative radiotherapy for rectal cancer were identified. The rectal tumour seen on the CT planning scan was outlined and measured in three dimensions. Surgery was carried out at a median of 42 days. The maximal cross-sectional area and volume of the tumour was recorded. Radiotherapy was delivered to a total dose of 50Gy over 25 fractions. The residual volume of tumour and degree of response to radiotherapy (grades 3,2,1) was assessed by histology. The tumour area and volume halving times were calculated. **Results:** The calculated pathological volume of the tumour ranged from 0 cm^3 to 45.8 cm^3 (median 4.2 cm^3). The mean cross-sectional area of the tumour following radiotherapy ranged from 0 cm^2 to 19.6 cm^2 (median 2.4 cm^2). ($p<0.0001$). The clinical response rate to radiotherapy was 87%. The median tumour volume halving time was 46 days with an average regression of 85%. Fourteen out of the 32 patients had a grade 3 response,17 patients had grade 2 and 1 had a poor grade 1 response. Multivariate regression analysis only the radiotherapy grading system was found to be significantly associated with extent of tumour regression ($p=0.026$). No other factors including age, sex, degree of differentiation of tumour, initial volume of tumour, site of tumour or interval between radiotherapy and surgery were significantly associated with regression.
Conclusion: The response rates to radiotherapy were in keeping with previously published data. The response grading system outlined provides a simple and predictive way of categorising response to preoperative radiotherapy.

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The Prevalence Of Helicobacter Pylori In Colorectal Neoplasms.

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Aim: To evaluate the presence of Helicobacter pylori (HP) within normal colonic mucosa and in different types of colorectal neoplasia.

Material and methods: Paraffin processed colonic tissue blocks of patients with a diagnosis of normal colonic mucosa (n=58), adenoma (n=59) and adenocarcinoma (n=59) were retrieved from our archives after being identified from the histopathology database. The adenoma group was further classified into tubular adenoma (n=19), tubulovillous adenoma (n=20) and villous adenoma (n=20). Anti-HP (Novocastra, NCL-HPp and NCL-C-jejuni) antibodies were used to identify helicobacter species in each of these cases.
Results: Significant numbers of helicobacter pylori were identified in tubular adenomas (OR=11.13; 95% CI=1.62-76.60), tubulovillous adenomas (OR=10.45; 95% CI=1.52-71.52) and adenocarcinomas (OR=8.13; 95%CI = 1.40-46.99) compared to control tissue but not within villous adenomas (OR = 2.95; 95% CI = 0.29-9.96)

Conclusion: HP has been implicated in gastric inflammation, ulcers, MALT lymphomas and adenocarcinomas. Similar aetiological associations with colorectal lesions have not been substantiated. We have demonstrated a significant association of helicobacter pylori presence with some, but not all, colorectal neoplasms. We can not however, infer a causal association between the bacteria and neoplasms nor can we exclude their presence as a consequence of the microenvironment created by the neoplasms.

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CA242 And CA50 Expression In Normal Colon And, In Colorectal Adenomas And Adenocarcinomas – Potential Tumour Markers?

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Aims: To assess phenotypic alterations in the expression of mucin antigens (CA242 and CA50) in colorectal neoplasia.
Methods: Formalin-fixed, paraffin wax embedded samples of normal colon (n=60), colorectal adenomas (n=60: tubular adenoma=30, (tubulo-) villous adenoma=30) and colorectal adenocarcinoma were retrieved from our archives and subjected to immunohistochemistry with CA242 and CA50. (Novocastra, UK; dil=1:100, microwave antigen retrieval).
Results: Positive immunohistochemical staining was noted at the luminal cell border, in Goblet cells, for a variable distance within epithelial crypts and in intraluminal mucus with both antibodies. CA50 staining was weaker, with less epithelial crypt cell positivity.
Upregulation of CA242 and CA50 was seen in tubular and (tubulo-) villous adenomas and in colonic adenocarcinomas; upregulation was also seen in hyperplastic areas adjacent to the carcinomas.
Both antibodies showed 100% sensitivity in detecting colonic adenocarcinoma, but showed poor specificity (68% and 53% respectively).
Conclusions: This lack of specificity precludes the use of CA242 and CA50 as tumour markers in colorectal neoplasia. Neither antibody was able to differentiate between adenoma subtypes and is therefore not able to be used as a marker of malignant transformation.

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The Impact Of Donut Histology On Patient Management: An Audit Of Compliance With Royal College Of Pathologist Guidelines.

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Aims: To audit donut histology and 1) compliance with Royal College of Pathologist guidelines, 2) impact on clinical management and 3) its relevance of the closest distal resection margin (DRM) in anterior resections (AR) for adenocarcinoma.
Methods: 158 consecutive AR histology reports were retrieved from the histology database between 2000 to 2003 (inclusive).
Results: These ARs were for benign disease (n=21), villous adenomas (n=7) and adenocarcinomas (n=130). Donuts were submitted with 76% (n=99) of AR specimens for adenocarcinoma; of these, 74% (n=34/46) of donuts were reported when the tumour was >30mm. 87% (n=46/53) were reported when the tumour was <30mm from the DRM but 13% (n=7) were not; in the latter instance, all DRMs were tumour free.
Nine (9%) of all submitted donuts (n=99) for adenocarcinoma were histologically abnormal; of these seven showed benign pathology and did not change clinical management. Two donuts were positive for adenocarcinoma but in these cases the DRM was also positive; the tumours were, in both instances, <30mm from the DRM and both operations were "curative". Subsequently, one underwent revision AR and the other an AP resection
Conclusions: There is, indeed, no need to examine donuts where the colonic tumour is >30 mm from the DRM but recommend that they are examined when the DRM is involved; clinical management was not altered in any of these cases.
Only in a small number of patients was clinical management altered on donut histology results; in both instances the tumour was <30mm from the DRM. We recommend that donuts must be examined where the tumour is <30mm from the DRM.

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Over-expression Of TCP1 Beta In Colorectal Cancers Correlates With Dukes Stage And Poor Survival

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Colorectal cancer is common in the Western World and has a moderate-poor survival rate with less than 45% of patients surviving five years after initial diagnosis. There is a need to identify molecular markers in order to improve prognosis and, to evaluate potential therapeutic targets. Using proteomic analysis, TCP1 Beta and Epsilon were identified as proteins which are over-expressed in colorectal adenocarcinomas. TCP1 Beta and Epsilon are subunit constituents of the TCP1 protein complex, which assists in the folding of actin and tubulin and has also been shown to interact with several other proteins such as cyclin E and the tumour suppressor protein VHL. We generated monoclonal antibodies to TCP1 Beta and Epsilon subunits, which were designed to work in formalin fixed, paraffin embedded sections. A colorectal cancer microarray was stained using immunohistochemistry. The presence of positive staining for TCP1 Beta and Epsilon and the cellular localisation of the staining were evaluated. TCP1 Beta was found to exhibit exclusively cytoplasmic staining. TCP1 Epsilon showed both cytoplasmic and nuclear staining. A trend was identified between increasing intensity of monoclonal antibody staining for TCP1 Beta and increasing Dukes stage (p=0.02). There was also a trend between increasing TCP1 Beta staining and reduced patient survival (p=0.07). These results suggest a role for TCP1 Beta in colorectal cancer progression.

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CLASICC Trial (Conventional Versus Laparoscopic-Assisted Surgery In Colorectal Cancer): First release of 3-Year Survival Results.

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This multi-centre randomised phase III trial was designed to compare conventional versus laparoscopic-assisted surgery for colorectal cancer. Three-year survival outcomes are planned for Summer 2005 and will be presented at the meeting.
Methods: Pathologists were trained at the start and 90% of pathology was centrally reviewed by PQ. Longer-term primary endpoints are local recurrences, disease-free survival and overall survival at 3 years; secondary endpoints are distant and port-site/wound recurrences.
Results: 794 patients were recruited, 526 to laparoscopic-assisted and 268 to open surgery, from 27 UK centres and 32 surgeons. Mean (SD) age 69 (11) years; 56% of patients were male; 20% of patients had pT1 or pT2 stage tumours, 56% had pT3 tumours and 15% had pT4 tumours; and 53% of tumours were node-negative. All patients have been fully followed up. Current median (range) follow-up is 30.8 (0.1-94.6) months, for patients still alive follow-up is 41.7 (1.5-94.6) months, and overall survival at 3 years currently stands at 69.6%.
Discussion: This will be the first release of the 3-year survival data from the trial. These results will provide valuable information on the pathological end points and longer-term survival outcomes following laparoscopic-assisted and open resection in colon and rectal cancer.

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Size Does Matter – Defining Adenoma Type. Testing The Hypothesis

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We have previously reported that flat and polypoid adenomas could only be separated once they achieved a certain size and below this size they should be called early adenomas as their future potential was unknown.

We have now sought to test this hypothesis on a further series of 85 adenomas collected since the first study and sought to investigate a population of serrated adenomas

Methods: 42 flat adenomas, 12 serrated adenomas and 31 polypoid adenomas were identified from the files and reviewed. All were digitised on an Aperio scanner and measurement made using imagescope software v6.7.

Results: Polypoid and flat adenoma populations diverged with increasing width but again a population of 'early adenomas were identified. All flat adenomas were below 1.3mm in height. Serrated adenomas generally followed polypoid adenomas in distribution but examples of flat serrated adenomas were apparent.

Conclusions: This hypothesis testing study confirms our previous findings of increasing divergence of flat and polypoid adenomas with increasing width and that flat adenomas do not appear to exceed 1.3mm in height. Early adenomas were again identified and this definition would be useful to introduce into practice. Serrated adenomas generally followed polypoid adenomas but examples of flat serrated adenomas were found.

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1Mb Resolution Array-CGH Identifies Small Chromosome Aberrations In Colorectal Cancers

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Colorectal cancers (CRC) show at least two patterns of genomic instability: chromosomal instability (identifiable as aneuploidy) or microsatellite instability (usually occurring in near-diploid CRC with inactivation of the DNA mismatch repair system). Sometimes both or neither type of instability coexists. Here, we have used a high resolution array with clones spaced on average 1Mb apart, for comparative genomic hybridisation (array-CGH) analysis to identify DNA copy number changes in over 80 colorectal adenocarcinomas. In addition the samples were analysed for their microsatellite status (by interrogation of 10 loci), expression of mismatch repair proteins hMLH1 and hMLH2 (by immunohistochemistry), DNA ploidy (by flow cytometry) and clinical and pathological parameters. FISH using a centromeric probe on chromosome 6 was used to adjust the copy number of the other chromosomes. As expected, large regions of DNA copy number changes were confirmed at the loci of genes known to show aberrations in CRC such as *APC* on chromosome 5*, SMAD4* on chromosome 18*,* and *p53* on chromosome 17. Both microsatellite stable cancers with gross aneuploidy, and near-diploid cancers with microsatellite instability showed small regions of DNA copy number change involving one or a few genes only. We found some previously undetected heterozygous and homozygous deletions of genes, as well as high-level amplifications of genes related to various signalling pathways. We conclude that, as well as chromosome and microsatellite instabilities, other small localised genomic abnormalities may afford selective growth advantage in colorectal neoplasia.

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Immunohistochemical Markers In Small Cell Carcinoma Of The Oesophagus And Lungs: A Comparison

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Introduction**:** Primary small cell carcinoma (SCC) of the oesophagus is a rare, rapidly growing, poor prognosis tumour. In the commoner lung SCC, several new markers are diagnostically significant. The oesophagus and lungs share a foregut origin and therefore might share immunohistochemical (IHC) markers.
Methods: IHC was performed on eight pairs of age-sex matched primary oesophageal and lung SCCs for thyroid transcription factor (TTF-1), CK7, CK20, CD56 and Chromogranin A (ChA).
Results:

	TTF-1	CK7	CK20	CD56	ChA	CAM 5.2
Oesophageal						
Positive	6 (75%)	6 (75%)	0	6 (75%)	7 (87.5%)	3 (37.5%)
Negative	2 (25%)	2 (25%)	8 (100%)	2 (25%)	1 (12.5%)	5 (62.5%)
Lung						
Positive	7 (87.5%)	3 (37.5%)	0	8 (100%)	5 (62.5%)	7 (87.5%)
Negative	1 (12.5%)	5 (62.5%)	8 (100%)	0	3 (37.5%)	1 (12.5%)

Conclusions: TTF-1, CD56, ChA and CK20 staining were similar, in keeping with the common embryological origin. CK7 and CAM 5.2 staining differed, the CK7 showing more positivity in the oesophageal tumours and the CAM 5.2 showing more positivity in the lung tumours. CK7 staining was stronger than CAM 5.2 staining. This may assist in differentiating between the two tumours.

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Total rectal and colonic expulsion with part of small bowel due to low flow phenomenon and non-obstructive bowel ischaemia

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We are reporting an unusually rare case where an elderly patient passed an extraordinarily long segment of bowel 1350mm in length, including the whole of the large bowel and part of the small bowel, through a vagino-enteric fistula following an episode of non-obstructive bowel ischaemia complicating a myocardial infarction.

Intestinal ischaemia is a devastating disease process that could lead to bowel gangrene and death if either not diagnosed early or left untreated; death is usually caused by irreversible shock, intestinal necrosis or septicaemia. It is usually seen in elderly patients with atherosclerotic disease. The course of bowel ischaemia may affect variable lengths of the intestine and it is not unusual for the condition to be followed by uneventful recovery. To our knowledge, there are only eight cases reported in the literature where the condition was diagnosed upon the passage of short segments of the large bowel particularly of the recto-sigmoid segment through the anus. This is the first case reported in which the bowel cast was expelled per vaginum.

We are also discussing the possible aetiological factors in this case.

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Epidermal Growth Factor Receptor (EGFR) Immunoprofiling in Colorectal Carcinoma – Paradoxical Nuclear Localisation

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Scoring systems for EGFR expression in colorectal cancer to predict response to anti-EGFR therapy emphasise membranous localisation but correlation between immunoprofile and response is weak. The best indicator is the severity of the skin rash during therapy.
The gene status and cellular localisation of the intra/extracellular domains of EGFR may be more relevant. We therefore assessed the expression of EGFR in 30 colorectal carcinomas (CRC) using antibodies to the extracellular and cytoplasmic domains.
EGFR was expressed in 28/30 CRC, with cytoplasmic expression of varying intensity. 6/28 cases showed membranous expression and 10/28 showed nuclear localisation.
In 20/28 cases cytoplasmic localisation was similar using both antibodies. In 2/28 there was no cytoplasmic localisation using the antibody to the extracellular domain and in 6/30 there was a difference in intensity between the 2 antibodies. Membranous localisation was detected mainly using the cytoplasmic domain antibody. Surprisingly, nuclear localisation was detected only on targeting the cytoplasmic domain.
The study shows it is not practical to use a single scoring system in all laboratories unless the technique is universally standardised. This is the first study reporting nuclear localisation of EGFR in sporadic CRC, and confirms our findings in adenomas and carcinomas from FAP patients. The significance of nuclear intracellular domain EGFR and its potential as a predictor for response to therapy need further evaluation.

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Gastrointestinal stromal tumour with acquired resistance to kinase inhibitor therapy associated with multiple secondary KIT mutations: radiological, autopsy and molecular correlation

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Initial enthusiasm over the success of imatinib mesylate in treating GIST has been tempered by the emergence of secondary resistance to treatment in many cases, from months to several years after initiation of treatment. Recent publications have described alternative molecular pathways to imatinib resistance in GISTs, with acquired *KIT* kinase domain mutations most common. In no case has more than one acquired mutation been described.
We describe a patient with malignant GIST who developed imatinib resistance and was enrolled on a trial of an investigational kinase inhibitor. After initial stabilisation of disease, she developed further progressive disease and died. *KIT* mutation analysis was performed on tumour samples taken: 1) before imatinib treatment; 2) after imatinib resistance developed but before treatment with the investigational kinase inhibitor was initiated; and 3) from different tumours at autopsy.
Mutation analysis revealed the acquisition of different *KIT* kinase domain mutations (D816H and V654A) in separate progressive tumours, in addition to the baseline exon 11 juxtamembrane region deletion. Non-progressive tumours demonstrated the baseline exon 11 mutation only and no progressive tumour harboured both acquired mutations. To our knowledge, multiple acquired *KIT* mutations in GISTs treated with kinase inhibitor therapy have not been previously reported.

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Correlation of Mismatch Repair Gene hMSH-2 and p53 Proteins Expression in Colorectal Cancer: An Immunohistochemical Study

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There is a sharp increase of colorectal cancer incidence in many countries in the developing world including Egypt; particularly in young people. Two pathways for colorectal carcinogenesis have been defined. The chromosomal instability (CIN) pathway and the microsatellite instability (MSI) pathway. Methods: To test both pathways, we examined 50 surgically resected colorectal cancer specimens, each entailing normal colonic mucosa and were immunostained for p53 and hMSH2 proteins expression. Results: Over expression of p53 protein was detected in 48% of cases; being more common in distal tumours (79 %) with significant reduced expression in mucoid carcinoma (p=0.02). Significant correlation with histological grade (p=0.002) was found, tumours of a higher degree of differentiation found are more likely to show p53 over-expression than poorly differentiated tumour.

Loss of hMSH-2 was found in 12% of cases, they were young (mean 25.6; median 28 years), tumours are predominantly of an expanding margin (p=0.06) and Duke's stage B (83.3%), with significant correlation with p53 protein expression (r= 0.327, p=0.028). Neither p53 nor hMSH-2 was correlated with age, gender and tumour site. The average age incidence of colonic carcinomas was 37.2±12.06 years. The tumours were common in the left than right side (2.1:1) with male to female ratio 1:1.2. CONCLUSION: In Upper Egypt; alterations of the p53 and hMSH2 proteins occur during colorectal carcinogenesis with quite similar characteristics to those of developed countries.

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Expression of Beta-Dystroglycan is Lost in Squamous Cell and Adenocarcinomas of the Oesophagus and This Loss is Significantly Related to Increasing Tumour Stage

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Dystroglycan is a protein with extracellular α and transmembrane β subunits which link the extracellular matrix and cytoskeleton by binding to laminin and other matrix molecules. Previously published studies have shown reduced expression of α-dystroglycan in many cancers but β-dystroglycan has only been investigated comprehensively by our group in prostate and breast cancer, this study investigates its expression in oesophageal cancer. 100 oesophageal cancers (24 squamous, 76 adenocarcinomas) and 20 samples of non-neoplastic oesophageal squamous epithelium and gastric body mucosa were arrayed in triplicate in tissue microarrays. Immunohistochemistry was performed using a monoclonal antibody raised against the cytoplasmic domain of β-dystroglycan. Staining was scored on triplicate cores and summated. β-dystroglycan was strongly expressed at both the intercellular and basement membrane junctions of non-neoplastic epithelial cells in the oesophagus and stomach. There was loss, or very weak, staining for β-dystroglycan at the intercellular junctions in 93% of the oesophageal cancers and basally in 82%. This loss of expression was significantly related to tumour stage (intercellular p=0.039, basal p=0.044) but there was no significant relationship with tumour type (0.791, 0.287), grade (0.667, 0.555), vascular invasion (0.862, 0.307) or lymph node status (0.885, 0.547). These results are similar to our findings in breast and prostate cancer. The absence of this important transmembrane protein in oesophageal cancer may play a significant role in tumour progression and the mechanisms for this require further investigation.

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Glucose-Regulated Protein 78 (Grp78) is Highly Expressed in Carcinoma of the Oesophagus, More Frequently in Adenocarcinoma than Squamous Cell Carcinoma

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Grp78 is a molecular chaperone that binds to malfolded proteins. It is upregulated under conditions of stress including glucose deprivation. *In vitro* cell line studies have demonstrated a correlation between increased expression of Grp78 and resistance to apoptosis by topoisomerase 2 directed drugs. There are no published studies investigating the expression of Grp78 in oesophageal cancer. In this study 79 oesophageal cancers (23% squamous cell and 77% adenocarcinoma) were sampled in quadruplicate in tissue microarrays, and immunohistochemistry for Grp78 (SantaCruz Biotechnology) was performed. The staining was scored semi-quantitatively with a cumulative total for 3 valid tissue cores from each case. There was strong (score ≥6) immunohistochemical expression of Grp78 in 84% of tumours. Oesophageal adenocarcinomas stained more strongly for Grp78 than did squamous cell carcinomas (p=0.001). There was no significant statistical relationship between expression of Grp78 and tumour grade (p=0.794) or stage (p=0.626). This study shows that expression of Grp78 is increased in the majority of oesophageal cancers and more highly expressed in adenocarcinomas. This expression may lead to a resistance to apoptosis-inducing drugs but its cell surface expression may provide a therapeutic target.

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Expression of S100A2, S100A4, S100A6 and S100A7 in Oesophageal Cancers Follows the Pattern of Expression in the Tissues From Which They Are Derived and Does Not Show Any Significant Association With Any Other Clinicopathological Parameters

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The S100 proteins are a group of proteins with functional heterogeneity but all share a similar structure with EF hand calcium binding sites. Their expression is altered in some cancers, including prostate and breast, but they have not been systematically investigated in oesophageal cancer. 100 oesophageal cancers (24 squamous, 76 adenocarcinomas) and 20 samples of non-neoplastic oesophageal squamous epithelium and gastric body mucosa were arrayed in triplicate in tissue microarrays. Immunohistochemistry was performed for S100A2, A4, A6 & A7. Staining was scored on triplicate cores and summated. S100A2 was strongly expressed in non-neoplastic squamous epithelium but not gastric body mucosa, S100A6 was strongly expressed in gastric body mucosa but not in squamous epithelium, S100A4 was expressed in neither, S100A7 was expressed in 2 cases of squamous epithelium but not in any other normal samples. This pattern of expression was carried into oesophageal cancers with squamous cancers expressing S100A2 and sometimes S100A7 and adenocarcinomas expressing S100A6. In all cases of squamous cancer which did express S100A2 or S100A7 this expression was limited to areas of obvious squamous differentiation and so these proteins are not useful markers of covert squamous differentiation. There was no significant relationship between the expression of any of these proteins and clinicopathological parameters including grade, stage, vascular invasion and lymph node status.

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There Is Loss of Expression of S100A8 and S100A9 in Oesophageal Squamous Carcinoma

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S100A8 (calgranulin A) and S100A9 (calgranulin B) are members of the EF-hand type calcium binding protein family which includes some proteins that have abnormal patterns of expression in human cancer. This study investigates their expression in oesophageal cancer. 100 oesophageal cancers (24 squamous, 76 adenocarcinomas) and 20 samples of non-neoplastic oesophageal squamous epithelium and gastric body mucosa were arrayed in triplicate in tissue microarrays. Immunohistochemistry was performed for S100A8 and S100A9. Staining was scored on triplicate cores and summated. S100A8 and S100A9 were both strongly expressed in non-neoplastic squamous epithelium in all cell layers except the basal. There was loss of expression of S100A8 in 22/24 squamous cancers and loss of S100A9 expression in 15/24. Since some squamous cancers still retained some expression there was a significant difference between expression in squamous and adenocarcinomas (S100A8 & S100A9 both $p<0.0005$). There were no other significant associations between expression of S100A8 or S100A9 and other clinicopathological parameters – grade (p=0.081 and 0.131), stage (p=0.722 and 0.925), vascular invasion (p=0.241 and 0.135), lymph node status (P=0.305 and 0.860). The loss of expression in the squamous cancers may be a reflection of the proliferative activity of the cancers (since neither protein is usually expressed in the basal layer of non-neoplastic squamous epithelium) or it could be due to hypermethylation of the promoter regions of the genes, a phenomenon that we have demonstrated in other S100 proteins in prostate cancer.

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Tissue Microarray Immunohistochemistry for S100A2, S100A4, S100A6, S100A8, S100A9, S100B and Annexin I With Unsupervised Cluster Analysis Produces a Useful Classification of Oesophageal Cancers

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The S100 proteins are a group of proteins with functional heterogeneity but all share a similar structure with EF hand calcium binding sites. Their expression is altered in some cancers, including prostate and breast, but they have not been systematically investigated in oesophageal cancer. 100 oesophageal cancers (24 squamous, 76 adenocarcinomas) and 20 samples of non-neoplastic oesophageal squamous epithelium and gastric body mucosa were arrayed in triplicate in tissue microarrays. Immunohistochemistry was performed for S100A2, A4, A6, A7, A8, A9, B and Annexin I. Staining was scored on triplicate cores and summated. The whole dataset was analysed using cluster analysis and a visualisation tool (Cluster & Treeview, EisenLab, University of California, US; de Hoon, University of Tokyo). There was clear clustering at the primary bifurcation of the hierarchical tree into non-neoplastic squamous epithelium, gastric body mucosa and oesophageal cancer with close to 100% accuracy. The oesophageal cancers subdivided further into those of squamous and adenocarcinomatous differentiation. There was no significant clustering into different tumour stages but the majority of these resected oesophageal cancers were late stage (pT3). This study shows that multiple protein expressions can classify oesophageal cancers into distinct grouping that correlate with known morphological patterns of differentiation. Selection of other proteins could produce a classification with new prognostic power.

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Cell Cycle and Oncogenic Protein Expression In Sporadic And Familial Duodenal Adenomas – An Immunohistochemical Study

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Introduction: The molecular pathogenesis of the adenoma-carcinoma sequence in the colorectum is well characterised but little is known about this in small intestinal carcinogenesis. We investigated this in sporadic and familial duodenal adenomas by measuring the expression of proteins involved in the adenoma-carcinoma sequence.

Methods: Twenty-eight (19 females) archival cases of sporadic duodenal adenomas (SDAs) and 25 cases of FAP adenomas were investigated by immunohistochemistry for: hMLH1, hMSH2, cyclin D1, beta-catenin, E-cadherin and p53.

Results

	p53 (%positive)	Nuclear beta-catenin (%positive)	E-cadherin (%positive)	cyclin D (%positive)
FAP (n=25)	52%	44%	36%	88%
Sporadic (n=28)	39%	25%	39%	100%

No cases were negative for hMLH1 or hMSH2. There was no statistically significant difference between protein expression in sporadic and FAP cases.

Conclusion: These results show some similarity to expression of the same proteins in colorectal adenoma. Beta-catenin showed some abnormal nuclear positivity in a quarter of the SDAs suggesting a possible role for the APC/beta-catenin pathway in their pathogenesis.

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Procoagulant Genes in the Pathogenesis of Inflammatory Bowel Disease and its Thromboembolic Complications.

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BACKGROUND:Patients with inflammatory bowel disease (IBD) frequently experience thromboembolic complications, which represent an important cause of morbidity and mortality. AIMS: To investigate the hypothesis that the prothrombotic state associated with factor V Leiden, prothrombin 20210A mutations and MTHFRC677T polymorphism is involved in the aetiology of IBD. PATIENTS AND METHODS:This study describes thrombotic markers in patients with IBD with (16 patients) and without a history of thrombosis (130 patients) relative to three sets of control populations, one inflammatory (non-IBD) control population (140 patients), one with a history of thrombosis (30 patients) and another healthy (90 controls). All samples and controls were from a 12-month period from a Caucasian Western European population who were unrelated. The study was further divided into prospective and retrospective studies. factors FVL, PT20210, MTHFR C677T were investigated by RFLP and Taqman- PCR. RESULTS and CONCLUSIONS:This study confirms that FVL mutation increases the risk for thrombotic events but is not more frequent with inflammatory bowel disease. The interaction of PT20210 and FVL suggests that the PT20210 should be included in thrombophilic testing. The study does not support the role of MTHFR in IBD except in patients with hyperhomocysteinemia. IBD is a risk factor for thrombosis when in the presence of other risk factors such as age. It was found that there was a higher prevalence of both FVL and PT20210 in inflammatory patients than normal healthy controls, which agrees with previous theories that inflammatory conditions have an increased risk of thrombosis. Finally, data extend and confirm the value of the TaqMan-PCR as a diagnostic tool for genetic screening of large cohorts for thrombophilic risk factors.

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Cytokeratins 7 and 20 In Gastrointestinal Tumours in Nigerians – An Immunohistochemical Study

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Introduction**:** The use of cytokeratins 7 and 20 in differentiating upper and lower GI tumours is well characterised in the developed world. However, there is little or no information on this in the developing countries such as Nigeria with a low incidence for such tumours. We investigated this using a series of GI tumours.

Methods**:** Eleven cases (6 males) of colorectal carcinomas (CRC) and 2 cases (both males) of gastric tumours were studied. The mean age of the CRC cases was 46years and both gastric cases were 65years old. The following immunohistochemical (IHC) markers were performed on paraffin-embedded blocks: CK7, CK20, CEA and MNF116.

Results**:**

CRC	CEA	MNF116	CK7	CK20
positive	11	11	3	2
negative	0	0	8	9
STOMACH				
positive	1	2	0	2
negative	1	0	2	0

CK7 and CK20 were positive in different cases.

Conclusions**:** The results suggest a difference in the staining pattern compared to the published literature. Approximately 30% of CRC are CK7 positive and another 20% CK20 positive. Majority of the CRC are negative for both markers. Also for the 2 gastric tumors are negative for CK7 but positive for CK20. These interesting findings needs further investigation with a larger series.

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Epstein- Barr Virus – A Case of Viral Associated Haemophagocytic Syndrome

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An 18 year old female presented, to Kings College Hospital, in February of this year with jaundice and a 10 day history of fevers and sore throat, for which she was given antibiotics, with no effect. She also complained of diarrhoea, vomiting and loss of appetite, for the last few days. She had previously been well, with only recurrent bouts of tonsillitis in her past medical history. Her umbilicus had been pierced three weeks previously, under sterile conditions. She had no other risk factors, which would explain her jaundice, and was on no other medication.

On examination she was conscious but apyrexial, with tonsillitis, very sore fissured bleeding lips and tongue, tenderness around the umbilicus, hepatosplenomegaly, and haematuria. Blood tests demonstrated a neutropenia and thrombocytopenia, with deranged liver and kidney function tests, suggesting overwhelming sepsis and possible leukaemia.

Antibiotics of Vancomyocin and Amikacin IV were started, as were transfusions of platelets, fresh frozen plasma and blood. Bone marrow aspirate showed a hypercellular bone marrow, with massive infiltration by macrophages containing numerous phagocytosed red cells, compatible with haemophagocytic lymphohistiocytosis and in situ hybridisation confirmed EBV infection.

By day 4 she had developed a rash on her limbs, consistent with DIC. In early hours of the morning on day 5 she had a cardiac arrest and passed away. An autopsy confirmed that death was due to EBV-associated haemophagocytic syndrome.

The aetiology, pathophysiology, clinical presentation and treatment of viral associated haemophagocytic syndrome will be discussed.

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Malignant Histiocytosis– Our Experience Of A Rare Tumour Presenting With Spontaneous Splenic Rupture

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Malignant histiocytosis (MH) is a rare invasive proliferation of neoplastic histiocytes. Cases previously reported as MH have been demonstrated to be lymphomas of T or B lineage, especially anaplastic large cell lymphoma. Regardless of site of origin, this tumor has an aggressive behavior and often presents with high stage disease. Although prognosis is poor, it may be potentially curable and a definitive diagnosis by immunohistological means is thus necessary to guide treatment. We describe a case of MH that presented clinically with symptoms suggesting pneumonia but then suddenly deteriorated and died. At autopsy, a large quantity of fresh blood originating from several ruptured nodules on the enlarged spleen was seen within the peritoneal cavity. Histologic examination of the spleen showed extensive infiltration by pleomorphic tumour cells and erythrophagocytosis by tumour cells. Immunohistochemistry was performed with the tumour cells staining strongly with CD68 and CD43. The tumour cells did not stain with T B cell markers, CD30, EMA, S100 protein and myeloperoxidase. To our knowledge, this is the second reported case of malignant histiocytosis presenting with spontaneous splenic rupture.

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Expression Of Oct2 And BOB.1 In Hodgkin Lymphoma (HL), Diffuse Large B Cell Lymphoma (DLBCL) And Anaplastic Large Cell Lymphoma (ALC)

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We investigated the pattern of immunoexpression of the B-cell transcription factors Oct2 and BOB.1 in Hodgkin lymphoma (HL), diffuse large B cell lymphoma (DLBCL) and anaplastic large cell lymphoma (ALC), and correlated this with CD20 and CD79a expression in HL and ALK expression in ALC.
Methods: Seventeen cases of classical HL, 3 nodular lymphocyte predominant HL (NLPHL), 20 DLBCL and 7 ALC were studied.
Results: All DLBCL and the L&H cells of NLPHL showed strong expression of both Oct2 and BOB.1. Oct2 was expressed by 8/17 classical HL and BOB.1 in 9/17; 7 cases expressed both factors. 6 HL cases expressed CD79a or CD20 and 5 of these co-expressed Oct2 and BOB.1. 5/7 ALC expressed BOB.1, 4 co-expressed Oct2. ALK was expressed by 2/5 cases, one of which expressed CD3.
Conclusions: BOB.1 and Oct2 are strongly expressed by DLBCL and NLPHL. Expression is also seen in half the cases of classical HL, supporting the B-cell origin of HL. Aberrant expression is seen in a large proportion of ALC of both null and T-cell phenotype. Positive expression of these markers is unhelpful in the differential diagnosis of HL and ALC.

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Counting CD34+ Cells in Bone Marrow Trephines of CML Patients – Which Method Correlates Best With Aspirate Blast Count?

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CD34 is expressed on early haematopoietic stem/progenitor cells. An increase in CD34 reactivity is observed in chronic myeloid leukaemia (CML). CD34 immunostaining in paraffin embedded bone marrow trephines is a reliable method of classifying phase of CML (chronic, accelerated and blastic) and thus contributes information on disease progression. Various techniques are described for evaluating CD34+ cells in trephines, however, the optimal method has not been outlined.
We studied 169 bone marrow trephines from 53 patients with CML who were being treated with imatinib. The immunostained sections were evaluated for percentage CD34+ cells in a minimum of 500 nucleated marrow cells, total CD34+ cells in 10 hpf and highest number of CD34+ cells in a single hpf. All methods were correlated with aspirate blast count (a critical parameter conventionally used in evaluation of CML phase). All showed significant direct correlation ($p<0.001$). However, the percentage count in 500 cells correlated best ($R=0.723$).
Both percentage CD34+ in 500 cells and aspirate blast count measure the relative proportion of precursors are therefore similar strategies. It remains to be seen whether volumetric measurements, assessing greater sample size, will prove to be better predictors of clinical endpoints compared to relative proportions.

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Langerhans Cell Histiocytosis-Like Lesions In Association With Other Lymphoproliferative Disorders: A Reactive Or Neoplastic Phenomenon?

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Langerhans cell histiocytosis (LCH) has been described in association with a variety of tumours – preceding, following or synchronous with the other tumour. It is possible that in some cases the presence of two pathologies is coincidental. However the incidence is much greater than would be expected by chance alone. The most intimate and perhaps interdependent relationship is between LCH and lymphoma, in which synchronous association is commonly seen. The nature of this association has remained elusive with debate as to whether a proliferation of LCs in this context represents a true clonal neoplasm, or an exaggerated reactive phenomenon. We have encountered five cases of LCH-like proliferations occurring in the context of other lymphoproliferative disorders. In the two female cases we combined laser capture microdissection with the human androgen receptor assay to determine whether the infiltrate of LCs was clonally restricted. A true nodal LCH was used as a control. Our results indicate clonal restriction of the LC population in the case of nodal LCH. However, the LC infiltrate seen in association with a pseudolymphoma and Hodgkins lymphoma is polyclonal, suggesting that at least in a proportion of cases, this is a reactive lesion, not a potentially aggressive second malignancy.

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Evaluation of Vascular Endothelial Growth Factor-A (VEGF) Expression in Follicular Lymphomas

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Vascular endothelial growth factor-A (VEGF) has been correlated with increased vascularity in tumours. Vascularization has been associated with improved clinical outcome in follicular lymphomas. The role of VEGF in these tumours is unclear due to conflicting reports and small, non-homogenous sample sets. We aimed to determine the expression pattern of VEGF in a larger, homogeneous sample set of follicular lymphomas.
We used immunohistochemistry (IHC) on formalin-fixed paraffin-embedded tissues (FFPE), and quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) on frozen tissues, to determine expression of VEGF protein and RNA, respectively, in follicular lymphomas (n = 9 frozen, n = 29 FFPE) and normal lymphoid organs (n = 4 frozen, n = 13 FFPE). Data from an in situ hybridization study will be presented at the meeting.
By IHC, 2/25 cases of follicular lymphoma, represented by cores on tissue microarrays, showed weak and focal immunoreactivity. Matched whole sections showed weak reactivity associated with small vascular channels in 8/29 cases; only areas involved by malignant lymphoma were considered. In benign lymphoid tissues weak staining was observed in the squamous mucosa of 2/3 tonsils and in the sinus-lining cells of the red pulp in the spleen. Normal kidney (positive control) was strongly positive in glomeruli, as expected. By qRT-PCR the mean expression of VEGF RNA was six-fold lower ($p = 0.018$) in follicular lymphomas (≥ 90% of tissue malignant) compared to normal lymph nodes.
VEGF expression appears to be down-regulated in follicular lymphomas compared to normal lymph nodes. This should prompt evaluation of additional indicators of angiogenic activity in malignant lymphomas.

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The practical use of PCR clonality analysis in lymphoma diagnosis: Experience from a large teaching hospital

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Background:
Clonality studies on the immunoglobulin heavy chain gene and T cell receptor gene by PCR are useful in cases of lymphoid proliferation where there is diagnostic difficulty but are not necessary in the majority of cases of lymphoma. Our aim was to determine the use of PCR clonality analysis in routine diagnostic procedure in UHL. Method:
We reviewed all new diagnoses of non-Hodgkin lymphoma from 2003 – 2004 and molecular analysis for B and T-cell clonality over the same period. Diagnoses and PCR results were subcategorised into different categories: site of tissue origin, reason for performing PCR, accordance of PCR results and conventional diagnostic methods, sufficiency of DNA extraction and amplification.
Results:
Lymphoma was diagnosed in 621 specimens (509 patients). Clonality analysis was performed in 118 of these specimens (102 patients). DNA amplification was successful in over 90% of cases. Analysis of skin specimens is especially hampered by poor DNA amplification. PCR is especially useful to reveal minimal residual disease. Our experience confirms the diagnostic utility of PCR but also documents its difficulties in certain specimens.

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P75+ve Bone Marrow Stellate Cells and Stromal Fibrosis in Chronic Idiopathic Myelofibrosis and Other Myeloid Proliferations

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A population of bone marrow stromal cells expresses the low affinity nerve growth factor receptor (p75) and shares other features with the hepatic stellate cell implicated in liver fibrosis. We hypothesise that p75+ve marrow stellate cells may be fibrogenic in myeloproliferative and myelodysplastic states.
An immunohistochemical survey of trephine biopsy sections from patients with such disorders revealed only sporadic cases with increased p75+ve cells in CGL, PV and subsets of primary myelodysplasia. No significant increase was found in 10 cases of ET while numbers of p75+ve cells were increased in all 15 cases of chronic idiopathic myelofibrosis (CIMF) studied. Unlike human liver stellate cells in cirrhosis, alpha-smooth muscle actin expression was only inconsistently increased in marrow stroma in CIMF and may be expressed by cells other than the p75+ve population. Semi-quantitative assessment of p75+ve cell numbers correlated with increased reticulin deposition but not with overt stromal fibrosis or new bone formation in CIMF. New bone formation also correlated poorly with the degree of fibrosis and with stromal angiogenesis, the latter measured by assessing CD34+ve microvessel density. However, in 7/10 cases, new bone formation was associated with retention of adipocyte differentiation by at least 20% of stromal cells. In long-term culture in vitro, p75+ve cells are the major source of adipocytes formed in marrow stroma. The role of p75+ve stellate cells in marrow fibrosis and new bone formation is clearly complex. The molecule provides a useful operational marker for further functional studies, with a view to therapeutic modulation of fibrogenesis in CIMF and other conditions associated with reactive marrow stromal fibrosis.

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A Rare Case Of A Retroperitoneal Dedifferentiated Mixed Liposarcoma

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Aim: To describe an unusual variant of liposarcoma with osteosarcomatous differentiation.
Case report: A 67-year-old female presented with a three month history of abdominal discomfort and intermittent pain in the left hypochondrium and flank, associated with loss of appetite and weight loss. A firm 150mm-diameter tumour mass was palpated in the left hypochondrium, flank and epigastric region. CT scan confirmed that this was a retroperitoneal tumour.
Macroscopically, the tumour was 260mm diameter and weighed 3093 gms. On sectioning it was yellow-white with extensive areas of mucoid degeneration and necrosis and, a 50mm diameter focal circumscribed white firm nodular area.
Histology showed a malignant soft tissue in which well differentiated and spindle cell liposarcomatous areas were identified; mild to moderate nuclear pleomorphism and mitotic figures were noted.
The sections from the circumscribed white area showed spindle to plump cells with intercellular collagen deposition and nodules of fibrocartilaginous tissue with areas of ossification and mineralisation. In addition, there was osteoid formation by the tumour cells in some areas. The cells were pleomorphic and showed mitoses. Focal collections of multinucleated giant cells were also seen.
Based on the findings, a diagnosis of a mixed liposarcoma with osteosarcomatous differentiation was made.
Conclusion: This is a very unusual and rare combination of mixed liposarcoma with osteosarcomatous differentiation in a retroperitoneal tumour.

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The Expression of Oestrogen Receptor alpha and Oestrogen Receptor beta Isoforms in Fibromatosis

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Introduction: The identification of a second oestrogen receptor (ERβ) has opened a new dimension into the investigation of its role in tumourigenesis, both in the breast and many other organs. Previous reports have shown that oestrogen receptor (ERα) is expressed in a small proportion of fibromatoses and hence treatment with antioestrogens has been attempted. No previous studies have reported on the expression of ERβ in those lesions
Materials and methods: Ninety-three specimens (from 80 patients) with the diagnosis of fibromatosis were identified within the Department of Pathology database over the last 5 years. Cases included palmar, plantar, mesenteric, chest wall, deltoid, thigh, penile and breast fibromatoses. Two cases of infantile digital fibromatosis were also included. The age of the patients ranged from 41 weeks to 90 years. Paraffin wax-embedded sections were stained with monoclonal antibodies for ERα, ERβ1 and ERβcx.
Results: ERβ1 and ERβcx were strongly expressed in the nuclei of the majority of studied cases (median 52% and 80% respectively). This expression was independent of the site of origin and the gender of patients. Only a few cases expressed ERα.
Conclusion: The two ERβ isoforms (ERβ1 and ERβcx) are predominant in fibromatoses of different tissues. The relative expression of both oestrogen receptors might have important implications in determining the response of those lesions to hormonal therapy.

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Vascular Endothelial Growth Factor A and Platelet-Derived Growth Factor Play A Central Role in the Pathogenesis of Digital Clubbing

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Digital clubbing is associated with many serious diseases but its pathogenesis is unknown. It has been hypothesized that platelet clusters impacting in the distal vasculature mediate the morphological changes of clubbing.
Since the multifunctional cytokines vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are released on platelet aggregation and are hypoxically regulated, we have examined their role in clubbing using immunohistochemistry. In addition to levels of expression of VEGF and PDGF, basic fibroblast growth factor (bFGF), transforming growth factor-beta 1 (TGF-β1), microvessel density, carbonic anhydrase IX (CAIX), hypoxia inducible factor (HIF)-1α, and HIF-2α were measured.
A significant increase in VEGF (p=0.01), pKDR (p=0.03), PDGF (p=0.017), and HIF-1α (p=0.004) and HIF-2α (p=0.004) expression together with a significant increase in microvessel density (p=0.03) was observed in the stroma of clubbed digits compared with controls. No difference was observed in CAIX (p=0.25), TGF-β1 (p=0.66) or bFGF (p=0.18) expression between affected and control groups.
These findings suggest that VEGF and PDGF are released after platelet impaction and that their expression is hypoxically enhanced after capillary occlusion. VEGF may synergize with PDGF in inducing the stromal and vascular changes present in digital clubbing.

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Epidermal Growth Factor Receptor (EGFR) expression and mutation patterns in high malignant central osteosarcomas

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The expression of the Epidermal Growth Factor Receptor (EGFR) in osteosarcomas has repeatedly been described. Experiences with anti-EGFR targetted therapies made clear that besides the expression status of EGFR, the knowledge about gene muations are of major, predictive power. We therefore aimed to explore the EGFR expression and gene muation status in high malignant osteosarcomas.
We investigated 55 samples of osteosarcoma patients of all age groups by means of immunohistochemistry, *egfr* Fluorescence in-situ hybridization (FISH) and quantitative real-time PCR targetting the first CA repeat in intron 1 of the *egfr* gene. 8 osteosarcomas were investigated additionally by means of Single Nucleotide (SNP) analysis for gene dosage alterations of *egfr* and the adjacent chromosomal regions.
68% of all osteosarcomas revealed an overexpression of EGFR. 7 out of 28 analyzable osteosarcomas showed *egfr* amplifications by means of FISH analysis. All these cases were EGFR expressing. 20% of all cases revealed deletions within intron 1 of *egfr*, whereas only 12% displayed amplifications within this locus.
A significant correlation between intron 1 mutations and EGFR expression could not be shown. No evidence existed for circumscribed amplifications of *egfr* by means of SNP-analysis. All results were independent of histological subtype and patient age.
In conclusion our result show that an expression of EGFR can frequently be observed in osteosarcomas and is due to a great variety of underlying genetic mechanisms. A further delineation of these mutations will be helpful for a better understanding of the pathobiology of osteosarcomas and a better targetted therapy prediction.

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A Real-time RT-PCR Method For Detection Of EWS/ATF1 Fusion Transcripts In Clear Cell Sarcoma Using Paraffin-embedded Material

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Clear cell sarcomas (CCS) frequently carry chromosome translocation t(12;22) resulting in EWS/ATF1 fusion transcripts. Detection of the abnormal transcripts using RT-PCR can help distinguish CCS from histologically similar entities such as some malignant melanomas. We have developed a real-time RT-PCR method for detection of EWS/ATF1 transcripts in paraffin-embedded samples.
A primer and dual-labelled probe real-time PCR set was designed to amplify and hybridise to short fragments spanning the most common EWS/ATF1 breakpoint. RNA was extracted from nine formalin-fixed paraffin-embedded (FFPE) samples of CCS with proven EWS/ATF1 translocations and from fifteen other sarcomas submitted for analysis without the translocation. cDNA was generated using an ATF1 directed anti-sense RT primer. After optimisation using cDNA derived from fresh tissue CCS samples, real-time PCR was carried out on all selected FFPE cases using a FAM labelled probe.
All nine CCS were positive with real-time PCR with C_T values ranging from 27 to 36, which was comparable to house-keeping gene analysis. The non-CCS samples were negative.
This real-time PCR method permits detection of EWS/ATF1 fusion transcripts in routine FFPE tissue samples with comparable results to conventional RT-PCR. The real-time approach offers the advantages of speed, reduced contamination risk and potential for quantification.

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Detection Of Ewing And Synovial Sarcoma Fusion Transcripts Using Conventional And Real-Time RT-PCR

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Diagnosis of Ewing (ES) and synovial sarcomas (SS) can be difficult and may be aided by detection of characteristic fusion transcripts (EWS/FLI-1 or EWS/ERG in ES and SYT/SSX in SS). We have compared standard with real-time RT-PCR, which is quicker, quantifiable and reduces contamination risk.

RNA was extracted from 102 samples submitted for molecular analysis (21 ES, 27 SS and 54 others) and subjected to RT-PCR for detection of EWS/FLI-1, EWS/ERG and SYT/SSX fusion transcripts with published standard and real-time RT-PCR methods using dual-labelled probes.

EWS/FLI-1 transcripts were detected in 15 cases (all ES; 71%) using conventional RT-PCR and in 14 using real-time RT-PCR. No EWS/ERG transcripts were detected. 27 SYT/SSX transcripts were detected in SS (100%) cases by both conventional and real-time RT-PCR. No fusion transcripts were detected in non-ES or non-SS cases.

Conventional and real-time RT-PCR can detect sarcoma type specific fusion transcripts in a high proportion of ES and SS. Identical results were achieved with conventional and real-time RT-PCR with SYT/SSX analysis, however there was one presumed false negative in the real-time analysis with EWS/FLI-1/ERG primers. As these methods can be applied to paraffin-embedded samples they offer a powerful aid to the diagnosis of these difficult tumours.

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Sequential sampling: optimising the use of two endoscopic ultrasound (EUS) guided tissue sampling techniques

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Background: Each of the two EUS guided sampling techniques; fine needle aspiration (FNA) and Tru-cut biopsy have advantages and limitations when used alone. We have previously shown that combining the two techniques would increase the accuracy of EUS- guided sampling, but this involved performing both FNA and Tru-cut biopsies in all the patients. **Aim**: To investigate whether performing FNA, only when Tru-cut biopsy samples were inadequate, would be a useful strategy in EUS-guided sampling.
Methods: EUS guided sampling and Tru-cut biopsies for FNA were performed. Maximum 4 passes were made for Tru-cut biopsy and if the samples obtained were inadequate, further FNA was carried out for cytology. We compared the results of this approach (sequential sampling) with 25 previous patients who had undergone both Tru-cut and FNA (dual sampling). **Results**: Thirty nine patients (8 females) aged 34-83 (median 66) who underwent "sequential sampling" were compared with 25 (9 female) aged 22-86 (median 63) who had "dual sampling".There were no significant difference in the maximum diameter of the lesions sampled in the two groups (0.5-6.0 [mean±SD 2.55±1.4] cm vs. 1.5-5.7 [3.38±1.2] cm) or the route of sampling (oesophagus/ stomach/ duodenum in 17/18/4 vs. 6/15/04). The number of passes made were similar (n= 2-7) in both groups [3.54±1.5 vs. 4.16±1.2] and there was no difference in the length of the tissue core (0-2 [1.19±0.6 vs. 0.89±0.5] cm). The diagnosis reached with sequential sampling was accurate in 36/39 (92%) cases compared with 23/25 (92%) in dual sampling group. Six out of 39 in the sequential sampling group needed FNA. **Conclusions**: Accurate diagnosis was reached in 92% of cases using "sequential sampling" strategy with 15% of the patients requiring both Tru-cut biopsies and FNA.

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Validation of the Aperio ScanScope® as a Tool in Routine Diagnostic Histopathology

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The Aperio ScanScope® is a high throughput system producing high quality virtual microscopy.
To validate the system, 45 consecutive breast core biopsies conventionally reported by a single consultant histopathologist in 2002 were re-reported by the same pathologist in 2005 using virtual images and access to the same clinical data. The B codes, tumour types and other significant reported features were compared with those originally reported.
The scanning time times were in the region of 15 minutes / slide with an average compressed file size of 115MB. Image access times were minimal. Reporting times were identical to using conventional microscopy.
In all but 2 cases the B codes were identical to those reported conventionally. The discrepancies were one B1 to B2 change and one B2 to B3 change. These changes were related to interpretations aided in the initial reporting by MDTM discussions, and unrelated to image quality. Two very subtle cases leading to a B4 code were easily recognised on the virtual slides. Invasive tumour types and presence and grading of concomitant DCIS were consistently reported on the virtual images.
The results indicate the Aperio virtual microscopy system can be used with confidence in the routine diagnostic setting.

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Quantum dot based in situ hybridisation for gene expression profiling.

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Quantum dots (QD) are fluorescent semiconductor nanocrystals (2-10nm) possessing the unique properties of extremely high fluorescence efficiency, lack of photobleaching and long fluorescence lifetime, making them ideal tool for bioimaging. We have developed a novel technique for in situ hybridisation (ISH) using biotinylated oligonucleotides conjugated to streptavidin coated QD, enabling their use for translation of microarray gene signature to tissue sections, in this study in bone marrow trephine samples.
The results demonstrate necessity of a low oligonucleotide to QD conjugation molar ratio, whilst the degree of saturation of binding sites per QD by oligonucleotides can be demonstrated by gel electrophoresis. The predigestion and hybridisation conditions for ISH have been optimised using a 50 base pair long poly T oligonucleotide, which shows clear cytoplasmic binding. Images obtained by fluorescence microscopy have been analysed using a multispectral imager (CRI, USA) enabling distinction of signal from autofluorescence.
Though this study has concentrated on trephine biopsy samples it is applicable to any other tissue type, whilst use of oligonucleotide probes and spectral imaging allows extension to multiple gene analysis. This method therefore provides a platform for translation of microarray signatures to tissue sections with the ultimate goal of molecular staging of cancer.

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A Simple And Cost-Effective Way Of Using Bioinformatics To Solve A Complex Microarray Data-Mining Problem In The Molecular Pathogenesis Of Lung Cancer

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The aim of the project was to simplify processing of data from a loss of heterozygosity (LOH) study of lung cancer, using Affymetrix 10K single nucleotide polymorphism (SNP) arrays. Data from the proprietary software GDAS (GeneChip Data Analysis Software) were exported into Microsoft Excel. A custom-designed macro then filtered the SNP data to flag candidate regions for LOH, tested the data for LOH based on SNP calls and finally charted the results by chromosome. Interesting SNP intervals can be exported into web-based tools to identify tumour suppressor genes in LOH regions. As more samples are genotyped, there is a corresponding increase in data generated. The use of a simple spreadsheet macro is an ideal way to efficiently use existing resources; manual processing is reduced significantly, without the need for further purchase of proprietary software.
We present this project as an example of applying existing resources to a complex data-sorting problem in a cost- and time-efficient manner.
The program is available for download at http://www2.warwick.ac.uk/fac/sci/bio/services/molbiol/research_projects/

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Tissue microarrays; application to quality control for immunohistochemistry

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Tissue micro-array (TMA) technology permits the simultaneous evaluation of immunoprofile of multiple normal tissues or different tumours. This can assure application of a standardised method that is cost effective and time saving.
We report the implementation of TMA blocks for use in a Quality Management Environment diagnostic service for immunohistochemistry. There are important considerations in creating a reference tissue microarray block composed of a variety of tumours and normal tissue. Such a block needs to show a range of levels of expression to a particular antibody.
The creation of blocks is labour intensive and may not be cost-effective for control of common tissues. However, there is a value in seeing a range of levels of immunoreactivity in a single block for validation of antibody batches and titration of appropriate dilutions for staining.
In extending the use of TMA in a routine laboratory we plan to look at two additional areas:
1) Looking at whether sections cut at different times might show a variable level of immuno-reactivity through the depth of a punch sample. This is in comparison to previous studies which have assessed two dimensional heterogeneity. An important consideration in using this technology compared to using conventional whole tissue sections concerns the risk of sampling a non-reactive area.
2) To assess the feasibility of using the levels of expression/immunoprofile of such a reference block when compared to patient samples as an aid to suggest a possible primary site of origin in cases of undifferentiated poorly differentiated metastatic carcinoma of unknown primary site.

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A Simple and Rapid Approach To The Problem of Tissue Contamination

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We present a case in which we strongly suspected that contamination of a specimen had occurred on the cut-up bench, and the objective evidence produced which confirmed our suspicion. In the suspicious section a tiny fragment of poorly differentiated carcinoma lay on the surface of benign colonic mucosa. The carcinomatous fragment was recognised as identical to the material present in the bladder tumour, which had immediately preceded the colonic biopsy on the cut. Paraffin blocks of both the ?contaminated tissue and the suspected contaminating tumour were retrieved from file and inspected. The suspected contaminating tissue could be identified with the naked eye. A blade was used to scrape the tissue to create 3 samples: (a) benign colonic mucosa only (b) benign colonic mucosa and ?contaminating tumour (c) tumour tissue from suspected contaminating bladder tumour. The DNA was extracted and PCR analysis carried out using a panel of 12 polymorphic microsatellite markers, specific for chromosomes 13, 18 and 21. These markers are routinely used for rapid trisomy screening. The results show clearly that the colonic mucosa from one patient was contaminated by bladder tumour from the other patient. There was no significant delay of the report and the error was not reported to the clinicians. This technique, with the use of more refined microdissection if required, can be used to sort out such rare, but inevitable, instances of contamination and prevent further unnecessary investigations of innocent patients.

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Digital Imaging Improves Specimen Through-Put in a Diagnostic Transmission Electron Microscopy Service

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Recently digital cameras have been able to produce resolutions comparable with photographic methods in both light microscopy and transmission electron microscopy (TEM).

Over a year ago, a Mega View III CCD camera system with SIS software was installed on our JEOL 1200EXII diagnostic TEM. The images produced have a greater dynamic range than photographs and are automatically calibrated following integration with the microscope. The data-rich image files are archived by the SIS software which provides reporting facilities and maintains a complete history of the specimen. Digital image field is restricted but it is easy to take a larger number of images to compensate. The camera is so sensitive that images of thin resin sections require only low beam settings, especially important for acrylics. There is no down time due to reloading of photographic plates and so the instrument is available without interruptions.

Images are stored on a central file server for pathologists to access by computer avoiding time-consuming and costly developing and printing. We process approximately 400 cases a year with a staff time saving of at least 800 hours per annum. Turnaround times for renal biopsies have been reduced to less than 4 days.

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The Interpretation of Fractures at Autopsy

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Bones tend to break in predictable ways and in predictable patterns. Leaving aside cases of child abuse, and with the exception of the skull, where bones are readily visible, pathologists tend to overlook the detail of most fractures.

Much can be learned from the anthropologists in understanding the basic mechanics of bone fracturing, and how these are dependent on the forces applied and on the strength and structure of that particular bone. Bones are not uniform, having areas of strength and areas of weakness.

Putting this into the forensic setting of falls, assaults, road accidents, firearms and child abuse, questions such as site of impact, direction of force, number of blows, and the age of an injury can begin to be addressed. While much of this is largely the province of the forensic pathologist, the general pathologist can expect to see people falling in the street, out of buildings, and in front of cars, and may be asked to interpret their findings.

In skeletonized remains, the finding of fractures offers the only means of identifying what happened to the individual, of major relevance as the victims of past war crimes increasingly become the subject of forensic investigation.

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MORPHOLOGICALLY BLAND VULVOVAGINAL MESENCHYMAL LESIONS : AN UPDATE

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Vulvovaginal mesenchymal lesions composed of morphologically bland spindle shaped cells often pose a particular diagnostic problem for the surgical pathologist, not only because of the rarity of these lesions but also because of the wide array of entities with overlapping morphological features. Included in this group of lesions are soft tissue neoplasms that may arise at any site and those that are characteristic of, or relatively specific to, the vulvovaginal region. Lesions that are relatively specific to the vulvovaginal region include well known neoplasms such as aggressive angiomyxoma and angiomyofibroblastoma as well as more recently described lesions such as cellular angiofibroma and superficial cervicovaginal myofibroblastoma (myofibroblastoma of the lower female genital tract). Fibroepithelial stromal polyp, superficial angiomyxoma and smooth muscle neoplasms also can occur at, but are not specific to, this site. In this talk, the clinical and pathological features of these lesions are described with an emphasis on recent developments. The value of ancillary studies, especially immunohistochemistry, is discussed, although it is stressed that in general these are of limited value and routine morphology remains the mainstay in diagnosis.

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Diagnostic Pitfalls In Smooth Muscle And Stromal Tumours Of The Uterus

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To assess malignancy in smooth muscle tumours of the uterus a range of features are needed including mitotic activity, tumour necrosis, pleomorphism, the nature of the margin and vessel invasion. The criteria used for variants of smooth muscle tumour differ. In the absence of atypia, mitoses up to 20 per 10 high power fields may not indicate malignancy and pleomorphic tumour cells occur in benign 'leiomyomas with bizarre nuclei'. Assessing outcome for epithelioid and myxoid smooth muscle tumours is difficult, even mitotic rates of 2 to 4 per 10 hpf may be a worrying feature in epithelioid tumours. Myxoid tumours, even with a relatively well-defined margin and low mitotic rate, may occasionally behave in a malignant fashion. Highly cellular smooth muscle tumours mimic stromal tumours. Assessment of the vascular pattern, cellular detail and the use of IHC are helpful. Haemorrhagic cellular leiomyomas are related to progestagen therapy and may mimic leiomyosarcomas. Embolisation of leiomyomas may also produce worrying changes. The relationship between intravascular leiomyomatosis and dissecting/non-dissecting cotyledonoid leiomyomas is not fully defined. Recently, 'pecomas' have been described in the uterus but epithelioid smooth muscle tumours may also show HMB45 positivity. The division of endometrial stromal tumours into benign stromal nodule, low-grade and high-grade stromal sarcoma is no longer accepted. Differentiation of LGSS from SN depends on the tumour margin. LGSS may show various patterns, including glandular and sex-cord-like. Tumours with stromal and smooth muscle differentiation behave as stromal tumours. LGSS may be difficult to differentiate from adenomyosis with loss of or sparse glands and adenomyosis with vessel invasion.

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Diagnostic Difficulties In Gestational Trophoblastic Disease

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The histological distinction between diploid androgenetic early complete hydatidiform mole and triploid partial mole is difficult because the classical features of complete mole diagnosed later in pregnancy may not be apparent. The following features are present only in early complete mole: polypoid configuration of chorionic villi, villous stromal myxoid change and villous stromal nuclear debris. Recent, hitherto unpublished studies, have shown the latter phenomenon to be the result of increased stromal cell proliferation and apoptosis. The differential diagnosis of complete and partial mole can also be assisted by the complementary use of ploidy analysis and immunohistochemistry for $p57^{kip2}$. The latter is a paternally imprinted, maternally expressed gene product absent in the villous trophoblast of complete mole. For reasons that are, as yet, not understood, non-villous (extravillous or intermediate) trophoblast expresses $p57^{kip2}$ in both complete and partial mole. The exuberant but physiological trophoblastic proliferation seen in the very early pregnancy of a tubal ectopic gestation may be misinterpreted as hydatidiform mole. The combination of complete hydatidiform mole co-existing with a non-molar fetus may be erroneously diagnosed as partial mole. There are a number of pitfalls in the diagnosis of neoplastic lesions of trophoblast, the commonest of which include: the erroneous diagnosis of placental site reaction and placental site nodule as placental site trophoblastic tumour and the erroneous diagnosis of the recently described epithelioid trophoblastic tumour as epithelioid leiomyosarcoma or even squamous cell carcinoma.

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PROBLEMS IN THE DIAGNOSIS OF ENDOMETRIAL HYPERPLASIA

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Diagnostic problems are encountered with every type of endometrial hyperplasia and as a consequence there are inter-observer differences.

Difficulties have been encountered in distinguishing prolonged proliferation and disordered proliferation from simple and complex hyperplasia and in distinguishing, on the basis of an endometrial biopsy, atypical hyperplasia from carcinoma which is invading the myometrium.

It can be argued that the only matter of importance to the clinician and to the patient is the risk of carcinoma, or the diagnosis of carcinoma. This is, however, probably the most difficult problem to resolve.

Some of the histological problems, including the recognition of metaplastic epithelium, and the recognition of histological features suggesting the presence of myo-invasive carcinoma will be illustrated and discussed and reference made to the appropriate terminology for such conditions.

The most minor abnormalities which might represent a neoplastic process in the endometrium will also be explored.

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Research Ethics: principles and current practice in the UK

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For many years ethical issues were the preserve of those involved in research based upon direct patient contact and intervention. However they now impact on all aspects of research and weigh heavily on the way in which pathological research is conducted. Tissue based research is now fully covered by an ethical framework based upon the principles of beneficence, non-maleficence, autonomy and justice. This framework is regulated in the UK by the Central Office for Research Ethics Committees
(COREC) established as a result of EU Directive 2001/20/EC. This presentation will overview the guiding principles and current mechanisms for research ethics.

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The Human Tissue Act: what it will mean for you

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The UK Government's reaction to concern about the use of post mortem tissue has led to the Human Tissue Act 2004. As might be expected, storing or using post-mortem tissue without consent will in most circumstances become a criminal offence. Unfortunately, the legislation goes far beyond the extent of the original problem, covering not just emotionally important post mortem tissue but anything which contains human cells - which includes blood, spit, urine and faeces.

The initial proposals (that the use of any human material without consent would be a criminal offence, even in quality control or apprenticeship-style teaching) have been tempered. But in most circumstances using human tissue in research will become a criminal offence if appropriate consent has not been obtained. Precise definitions of ‘appropriate consent’ are as yet lacking.

Other problematic provisions include the requirement for licensing, which although not yet fully defined is likely to mean that storing tissue for future research without a licence is an offence, even if the "tissue" is no more than a urine sample.

The belief of Ministers that clinical staff will willingly, repeatedly and with no remuneration seek patient consent to facilitate work in which they have no personal interest has yet to be validated.

The parallel imposition of cumbersome research ethics procedures designed originally for clinical trials has added to the inhibition of research in pathology.

The Act must be reviewed when the HTA and the HFEA are merged in 2008.

Research which does not happen is invisible to society. If pathologists are to influence the arguments they must understand the ethics and collect evidence. Objections without logical argument and evidence will carry no weight.

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The Public Face of Pathology

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The true face of pathology is not just invisible to the public, it is masked by myth and misinformation. The "organ retention scandal" reinforced, in the public mind, the association of pathology and pathologists with death investigations. However, most of the pathologist's work directly benefits living patients. The Department of Health (England) estimates that 70% of diagnoses in the NHS depend on pathologists. Patients meet their general practitioner and, if needed, a surgeon or other specialist, but they rarely meet the pathologist who often makes the most important medical decision — the diagnosis — affecting their care.

Why is public knowledge of the true role of pathology important? What benefits would result from improvements in public knowledge about the work of pathologists? Discord between public beliefs about pathology and the realities of its practice leads to tension and sometimes distress on both sides. When doctors campaign nationally or locally for more investment in some aspect of the health service, public support can be very influential in achieving a good outcome. Pathologists need public support for their work to advance medical knowledge; the public needs pathologists to lead improvements in healthcare.

Recent events exposed a firm bedrock of public support for tissue-based research. Bereaved parents distressed by unconsented "organ retention" invariably say that, if asked, their agreement for retention would have been given willingly. Pathologists are the custodians of tissues and organs donated for research. Pathologists can enable the altruism of those who donate organs and tissues, so that good can come from grief.

Pathology is the science behind the cure. Even post-mortems save lives.

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Approaches to Liver Tumour Resection

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Advances in surgical techniques have improved major liver resection for primary and metastatic tumours. "Anatomical resections" yield segmentectomy specimens (1 or more of 8 liver segments defined by vascular anatomy (IHPBA)), preferred to atypical (or wedge) resections because of better clearance and less bleeding. The regenerative capacity of remaining normal liver enables up to 80% functional parenchyma to be removed with the tumour. Most resections are for metastatic colorectal carcinoma (70% in Leeds) also for hepatocellular carcinoma (HCC) in non-cirrhotic patients and intrahepatic or hilar cholangiocarcinoma (CC) (each about 10%). Pre-operative tumour biopsy upstages disease and is avoided in potential surgical candidates.
The extent of most intrahepatic tumours is obvious naked eye. I paint the resection margin of the specimen, slice in horizontal plane for correlation with imaging, and record number of lesions, site, size, and distance from surgical margin, with histology for tumour characteristics, clearance, and background liver histology.
Metastatic colorectal carcinoma (unlike most primary sites) tends to have small numbers of large metastases suitable for liver resection, with 5year survival of 46%. Minimum clearance where possible should be >1 mm. However margin recurrence developed in only 29% with positive resection margin, probably because the surgical ultrasound dissector destroys tissue at the resection margin. Resection is possible in the minority of HCC without cirrhosis; tumour size, macroscopic and microscopic vascular invasion and state of background liver are the most important prognostic features.

For CC at the porta hepatis (Klatskin tumour) the extent of CC cannot be distinguished naked eye so all the resected extra-hepatic biliary tree is embedded in serial transverse sections. The high frequency of positive resection margins, perineural invasion, lymph node micrometastases and peritoneal involvement gives these tumours a poor prognosis but radical surgery is improving results.

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Vascular Tumours

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There is a spectrum of vascular neoplasms in the liver. Haemangiomas represent the most common benign hepatic tumour and indeed the liver is the most common site within solid organs for such lesions. These are generally single lesions that can undergo fibrotic change, become sclerosed and on occasions may become necrotic (so-called solitary necrotic nodule). Rarely multiple lesions involve the entire liver (diffuse haemangiomatosis). Infantile haemangio-endothelioma is a vascular tumour occurring during the first year of life; it may also be single or multicentric. It can be associated with other congenital abnormalities and may give rise to serious intravascular complications leading to bleeding diatheses. This tumour has generally been regarded as a benign neoplasm but there is now thought to be a spectrum which includes low grade angiosarcomas, some of which may metastasise.

Angiosarcoma is the most common primary mesenchymal tumour of the liver. This is a highly aggressive tumour. There are well recognised environmental factors which are thought to predispose to angiosarcoma, including exposure to Thorotrast, vinylchloride and arsenicals. Epithelioid haemangio-endotheliomas are low grade malignant tumours that have a characteristic growth pattern with obliteration of intrahepatic vessels. It may be complicated by a Bud-Chiari like syndrome. It is now recognised that there are some malignant vascular tumours in the liver that show features of both angiosarcoma and epithelioid haemangio-endothelioma. Finally, there are vascular abnormalities that give rise to tumour-like lesions, most notably hereditary haemorrhagic telangiectasia and peliosis hepatis.

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Paediatric Liver Tumours

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The identification of distinct paediatric epithelial liver neoplasms was an important achievement and has resulted in a so far useful classification. In ongoing international trials, an increasing spec-trum of novel paediatric liver cell tumours emerged, forming a complex family of lesions requiring a new classification approach.

In the group of undifferentiated hepatoblastomas, so far charac-terized by the small cell variant, tumours composed of large cells exist, similar to large cell types of medulloblastomas and neuro-blastomas. Liver cell neoplasms with a mature-looking hepato-cellular phenotype in young patients not only comprise macro-trabecular hepatoblastoma, but also the recently recognized transitional liver cell tumour. A further group of lesions is formed by stromal-epithelial and stromal neoplasms of the liver. Some hepatoblastomas exhibit a bimodal differentiation, including hepatoblastomas with cholangioblastic features and ' ductal plate tumours '. Finally, organoid liver tumours seem to mimick normal hepatogenesis.

The significance of these novel phenotypes will have to be measured in relation to their prognostic impact within a clinical setting, and to be tested in the circle of specialized pathologists in order to find an agreement for later clinical use.

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Gene Expression Profiling And Classification Of Adenocarcinoma By Site Of Origin

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Metastatic adenocarcinoma of unknown origin is a common clinical problem. Optimal patient management is facilitated by knowledge of the primary site. Unfortunately, metastatic adenocarcinomas have a similar histological appearance and so additional diagnostic markers are needed.

Over the past decade, the study of disease has been revolutionised by novel large-scale technologies. At the mRNA level, there are gene expression profiling and its analysis, that is, bioinformatics. Such data are made more powerful by their public availability. We have shown that the gene expression profiles of the seven most common adenocarcinomas differ according to site of origin. We have gone on to identify the differentially expressed genes and to select the best candidates for further study.

Large-scale screening at the tissue level can be achieved with tissue microarrays and immunohistochemistry. Using these, 27 candidate markers were profiled in 352 tumour samples. From this first round of testing, a classifier was developed containing ten candidates in a diagnostic table and decision tree. Their application to the original data correctly predicted the primary site in 88%. This figure was maintained in a second round of 130 independent primary and metastatic tumours. This classification scheme, alongside those already existing, should enable improved prediction of primary site in patients with metastatic adenocarcinoma of unknown origin and so their optimal therapy.

The application of genomic technologies to important clinical questions is yielding many candidate genes and gene panels. Pathology is central to their successful translation from bench to bedside; and pathologists are ideally placed to lead such research.

This research is funded by Cancer Research UK.

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The modern management of the patient with breast cancer. A celebration of the role of the pathologist.

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Over the last 30 years there have been dramatic improvements in the diagnosis and management of patients with breast lesions. Per-operative frozen section, an inherently inefficient procedure,has been replaced by fine needle aspiration cytology and needle core biopsy. The introduction of mammography and ultrasound, together with automated core biopsy and vacuum assisted mammotomy has led to **pre**-operative diagnosis rates for carcinoma in excess of 90% allowing proper counselling concerning therapeutic options.Increasing demand for conservation surgery and a widening range of systemic adjuvant treatments including hormone and cytotoxic therapy have all led to a search for prognostic and predictive factors. It has been established beyond doubt that traditional histopathological factors such as tumour size, histological grade, lymph node stage and lymphovascular invasion are the most useful factors for stratifying patients into prognostic groups. Assessment of hormone receptor proteins using immunohistochemical techniques, also the preserve of the histopathologist, has become an important therapeutic predictive factor.Histological type has been shown to make an important contribution to our understanding of the biology of breast cancer; for example, the medullary-like phenotype combined with P53 and CK14 positivity, ER and CerbB-2(HER2) negativity strongly predicts for BRCA-1 gene mutation carrier status. These factors have been incorporated into a Histopathological Minimum Data Set; the publication of National Reporting Guidelines has led to substantial improvements in consistency and reproducibility in their assessment. More predictive factors are needed and these will almost certainly be protein based biological markers, similar to the use of ER and HER2 for Herceptin therapy.

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Approaches to neck dissections

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The most important prognostic features that should be identified in neck dissection specimens are the number and anatomical location of nodes containing metastatic malignancy and the presence of extracapsular spread of tumour from the nodes into the surrounding tissues.

In order to accurately record these data, the surgeon and pathologist should have an agreed method for the orientation of specimens and indicating node levels; the use of labelled diagrams or metal tags is helpful. Specimen photography to indicate the sites of block selection is often useful. The pathologist needs to appreciate the anatomical relationships between the structures of the neck and that the separation of jugular nodes into groups is an artificial designation for what is, in practice, a continuous chain of nodes.

Lymph nodes should be identified by inspection and palpation. Nodes that contain obvious malignancy should be processed to include extranodal tissue. Other nodes should be sliced (if sufficiently large) and the entire node processed. Remember that even small nodes (<3mm) diameter may contain micrometastases. One haematoxylin and eosin-stained section is sufficient for routine diagnostic work. It is only essential to sample the submandibular salivary gland, jugular vein and sternomastoid if these structures are obviously involved by tumour.

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Thyroid Carcinogenesis
Genotype-Phenotype Correlation and the effect of Radiation

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Present knowledge of tumour behaviour and appropriate treatment is firmly based on a morphological classification. Current rapid advances in identification of genes and their mutations involved in carcinogenesis provide an alternative approach to tumour classification, and an alternative guide to appropriate therapy. In some tumours there is a close correlation between molecular and morphological classifications, in others the situation is more confused. Thyroid tumours include a range of well-defined morphological types derived from the follicular cell, as well as less distinctly demarcated subtypes. The relationship between morphological and molecular features of thyroid tumours will be discussed.

Knowledge of thyroid tumours has been enhanced by studies of the consequences of the Chernobyl accident, particularly the occurrence of large numbers of thyroid carcinomas in those exposed as children to high levels of radioisotopes of iodine in fallout. These thyroid carcinomas form the largest number of tumours of one type due to one cause at a known date that have ever occurred, and the interaction between the type of mutagen and the subsequent type of tumour developing will be presented.

Finally the advantages and disadvantages of either combining or separating morphologic and molecular diagnosis will be considered.

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SPECIALISATION IN CELLULAR PATHOLOGY – AGAINST

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The traditional pattern of "central" University Teaching Hospitals and "peripheral" District General Hospitals (DGH) is evolving with changes in cancer and other service provision and greater involvement of DGHs in medical student training.
There is a perceived wisdom that histopathology should become a fully specialised service along the lines of other major clinical disciplines.
This presentation will address the benefits to the health service, to pathology as a speciality and individual pathologists of retaining general histopathologists and cytopathologists in a district general hospital setting.

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Multidisciplinary teams improve the care of patients with cancer: the case against.

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Multidisciplinary team (MDT) working and, in particular, participation in multidisciplinary team meetings (MDMs) has been imposed on professionals caring for patients with cancer as a consequence of the National Cancer Plan. The evidence that MDTs improve clinical outcomes or clinical decision-making is scanty and contradictory in all forms of medicine. The evidence to support MDT working in cancer is almost non-existent. There is no evidence that decisions made by different MDTs are consistent or even that any one MDT makes consistent decisions on patients with similar clinical characteristics.

The drain that MDMs impose on human resources is significant, a fact that has been highlighted by the job-planning exercise that has accompanied the new consultant contract in England. This has not been resourced and imposes a disproportionate burden on those specialities whose members may be called upon to attend multiple MDMs for different tumour types, such as histopathology, radiology, and clinical- and medical- oncology. More than 10% of the consultant work-force may be occupied in, or travelling to, MDMs.

For many years the accepted wisdom in the world of business was that team working produced superior results. Recent thinking in this sphere questions this supposition and there is an emerging trend towards encouraging individuality and individual working. Having only latterly accepted the "wisdom" of team-working, medicine may once again find itself travelling in the opposite direction to the commercial world.

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Autopsy practice should be an essential part of postgraduate training in pathology - for

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There can be few, if any, medical practitioners who entirely happy with every aspect of their professional life. Histopathologists enjoy an ordered pattern of working, a wide variety of clinical material and the fascination of microscopy in a surgical setting. The lack of direct patient contact and post mortem examinations are cited as the major drawbacks of our speciality. All histopathologists are aware of the large contribution that autopsy pathology has made in recent years, especially in infectious disease and cardiac and neuropathology. It is inevitable that further advances will be made in the next decade. Yet if today's motion is carried will we have young consultants with the necessary training and expertise?
Many factors have conspired to diminish the profile of post mortem pathology and the consequent decision to invent the autopsy free pathologist. Facilities are usually poor, even in large teaching centres. In many it is impossible to perform examinations with a precision that should approach a surgical procedure.
Pathology has lost its prominent place in most undergraduate curricula and post mortem demonstrations are poorly attended. Forensic pathology has been largely privatised and some Coroners place restrictions on how post mortems should be performed.
In debate it will be argued that the solution is an improved and more detailed training and assessment in autopsy practice. I will urge CPA inspectors to demand appropriate investment in post mortem facilities. I will show how structured training courses in autopsy pathology consistently attract large numbers of pathologists in training. Finally evidence will be produced to demonstrate that the lure of autopsy free curriculum has not increased the number of UK/EU graduates applying for pathology training.

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Autopsy practice should be an essential part of postgraduate training in pathology – Against

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A survey in 2003 of SpRs found that only 75% wished to continue with autopsy experience
If the proposed reforms of the Coroners and Death Certification systems come into force, the number of medico-legal autopsies in England & Wales & NI could halve from current 120,000 pa.
The largest reduction in this workload may be in those cases - deaths in the community - that traditionally have demanded less detailed or lengthy autopsy examination to reach a not-unnatural cause of death.
The College is now defining adult-autopsy-active pathologists as those who perform >50 cases a year.
There will be increasing demand from clinicians and coroners for improved quality in the product of the autopsy, i.e. addressing the issues raised by a death to the satisfaction of all interested parties.
There is a continuing trend for specialisation in autopsy practice, with certain scenarios referred to centres (infection, maternal death, post-operative deaths)
If – over decades – we move to the Australian system where all non-perinatal autopsies are in the province of the forensic pathologists, there will be a further disincentive to train in good general autopsy practice.
Autopsy pathology and current histopathology/ cytopathology have little common overlap in practice.
Conclusion: some basic autopsy experience for 1-2 years into pathology training, then drop autopsies or take them seriously and specialise.

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Perplexing Patterns, Perspicacious Pathologists: Reflections on the Diversity of Gonadal Neoplasms and those who have described them

{P} RH Young
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In this lecture tribute will be paid to those whose contributions have been pioneering in the field of gonadal pathology. This will be followed by a selective coverage of a few of the many patterns and cell types gonadal neoplasms may exhibit and the diagnostic problems that result. Knowledge of testicular tumors traces back to Percival Pott, Sir Astley Cooper and Thomas Blizard Curling, the latter two each writing books on the testis. The description, in 1906, by the French urologist Maurice Chevassu, of the seminoma, and the 1946 paper of Nathan B. Friedman and Robert A. Moore, which segregated out as a distinct entity embryonal carcinoma, represent, however, the pillars for the current classification of testicular tumors. In 1946 Pierre Masson described the spermatocytic seminoma and subsequently Robert E. Scully wrote the first English language paper on that neoplasm and described several subtypes of sex cord tumor, the distinctive gonadal lesion, the gonadoblastoma, and played the major role, in 1980, in formulating the current classification of premalignant lesions of the testis. The British School under the leadership of R.C.B. Pugh and others contributed greatly to our understanding, first in 1962 in a special supplement to the British Journal of Urology, and then in a textbook published in 1976.

Three legendary figures, Thomas Hodgkin, Richard Bright, and Sir James Paget were among the first to write extensively on ovarian neoplasms. In 1870 Heinrich Waldeyer, and later in that century, another German, Hermann Johannes Pfannenstiel wrote important papers on the surface epithelial tumors. The latter was likely the first to refer to neoplasms now known as of "borderline malignancy" and also wrote on pseudomyxoma peritonei and other topics. Their countryman, Robert Meyer, subsequently recognized the Brenner tumor as a distinctive neoplasm and proposed the first classification of Sertoli-Leydig cell tumors (arrhenoblastomas). He also coined the term "disgerminoma" (soon changed to dysgerminoma) for the ovarian tumor that had been described in detail by the French investigator Marcel Chenot in 1911. During the Meyer era other significant contributions were made by, amongst others, Howard C. Taylor writing on the borderline tumors and John A. Sampson writing on endometriosis and tumors associated with it.

Major contributions were made later by Gunnar Teilum of Denmark and Lars Santesson of Sweden. Teilum delineated the nature of the yolk sac tumor (in both gonads) after noting the resemblance of papillary formations within it to the endodermal sinuses of the rat placenta and described most of its patterns. Santesson played a major role in formulating the first organized classification of the surface epithelial-stromal tumors of the ovary and also promoted the endometrioid carcinoma as a special variant of ovarian cancer. In a career spanning almost 60 years, Robert E. Scully described many now famous ovarian tumors including the juvenile granulosa cell tumor, small cell carcinoma of hypercalcemic type, retiform Sertoli-Leydig cell tumor, sclerosing stromal tumor and sex cord tumor with annular tubules. He was the driving force behind the influential 1973 World Health Organization classification of ovarian tumors and his later book "Tumors of the Ovaries and Maldeveloped Gonads" utilized that classification and presented a lucid elaboration of his by then vast experience with ovarian tumors. That work had been preceded by a short interval by another outstanding book "Tumours of the Ovary" by Professors Fred A. Langley and Harold Fox, doyens of British gynecological pathology from the illustrious Manchester School. It used the same classification and these two books helped greatly improve diagnosis and, accordingly, treatment.

The great efforts of those mentioned above, and many others, have brought order to the approach to diagnosing gonadal neoplasms which because of their greatly overlapping patterns and cell types can present a complex riddle from which the correct diagnosis must be deduced. Despite the over exaggerated claims of the real, or potential, role of new techniques in assisting in this endeavor, attention to basic principles of anatomic pathologic, clinical background, gross evaluation, thorough sampling, and awareness of the patterns and cell types tumors may exhibit, and associated features that favor one or the other diagnosis, are the bedrock to the approach that will bear fruitful results. Selected patterns and cell types that are particularly interesting to explore are considered based in large part on a prior essay (Seminars in Diagnostic Pathology 18:161-235, 2001). Follicular neoplasms are first discussed, beginning with two distinctive tumors, exclusively, or largely, encountered in the young, the juvenile granulosa cell tumor and small cell carcinoma of hypercalcemic type. Although follicles, and in some cases cells with abundant eosinophilic cytoplasm, are shared features it will be shown that the overall features of these neoplasms are distinctly different. Problems caused in the diagnosis of these two neoplasms by the occurrence of spacing resembling follicles in other lesions including even metastatic neoplasms will be noted. The follicles in the aforementioned are typically large but attention will then turn to neoplasms with small follicles or small spaces that may be mistaken for follicles, using as the "anchor" the famed Call-Exner bodies of the granulosa cell tumor. Other tumors may have spaces of similar caliber, particularly the endometrioid carcinoma, transitional cell carcinoma and carcinoid tumor. Associated features of diverse types should, nonetheless, readily distinguish these neoplasms. Confusion caused by the overlap in cell types is next discussed using as example certain issues related to small cell tumors, oxyphilic cell tumors and clear cell tumors. Gonadal tubular tumors are then discussed based on the prototypical tumor in this category, the Sertoli cell tumor of the testis. The presentation will conclude with a consideration of tumors with slit-like spaces appropriately linking famous neoplasms such as the serous carcinoma, yolk sac tumor, and retiform Sertoli-Leydig cell tumor, and exemplifying well some of the fundamentals of gonadal tumor evaluation which the lecture will have attempted to highlight.

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Companion Meeting: Thursday 7 July 2005

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Approaches to BMS dissection and sampling of breast specimens – the Nottingham City Hospital experience

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The role of biomedical scientists in breast specimen dissection and sampling will be described, drawing upon the experience of a senior biomedical scientist and consultants from the Nottingham City Hospital. The importance of defined working practices, and training systems will be highlighted. Particular emphasis will be placed upon the need for cooperation between biomedical scientists and consultant pathologists when developing a program for training both biomedical scientists and junior pathologists in the dissection and sampling of breast specimens.

ACEM ABSTRACTS

ASSOCIATION OF CLINICAL ELECTRON MICROSCOPISTS

COMPANION MEETING

Thursday 7 July 2005, Newcastle-upon-Tyne

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A Histological And Ultrastructural Study With Microanalysis Of Hysterectomy Specimens For Failed Laser Endometrial Ablation

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The treatment of menorrhagia can be by endometrial ablation. Curettage, diathermy, Nd:YAG laser, and thermal balloon ablation, are all options.

This study is of eight hysterectomy specimens, for failed Nd:YAG laser endometrial ablation; by conventional histology, transmission electron microscopy and x-ray microanalysis. Whilst a number of studies have been carried out histologically, no systematic ultrastructural examinations have been reported.

At the endo-myometrial junction, foreign body granulomas were present. The giant cells were reacting to lakes of brown material with admixed black granules. In three of the cases, in addition, lakes of optically clear intensely birefringent material was found.

By electron microscopy all of the foreign body granuloma related material was electron dense; both the brown and black material were full of tiny holes whilst the birefringent material was more homogenous and vaguely lamellar.

X-ray microanalysis of the brown and black material revealed peaks for mainly phosphorus and sulphur with smaller amounts of iron and calcium. The birefringent material was similar to the background.

In conclusion, analysis of the brown and black material showed that it is most likely to be remnants of charred tissue whilst the birefringent material is mostly likely to have come from the fibre-optic probe's plastic sheath.

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From the Basement Up – Ultrastructural Clues in Hereditary Skin Disorders Epidermolysis Bullosa and Ichthyosis

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From the basement membrane to the stratum corneum and from the basement microscopy lab to the molecular genetics lab, electron microscopy (EM) still plays an essential part in the diagnosis of many forms of epidermolysis bullosa (EB) and ichthyosis.

EM and immunofluorescence microscopy are used to investigate the ultrastructure and immunohistochemistry of skin biopsy samples in order to distinguish types and subtypes of these disorders. Moreover, examination of fetal skin biopsies using these techniques also enables prenatal diagnosis of some forms of EB and ichthyosis. Despite enormous progress in understanding the molecular genetics of many inherited skin diseases, microscopy still has a key role to play in identifying appropriate genes for mutation analysis and in identifying clues to candidate genes in unclassified disorders. Co-ordinated collaboration between clinicians, microscopists and molecular geneticists continues to lead to new understanding of genotype-phenotype correlation and the recognition of previously undescribed conditions.

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Ultrastructural Telepathology – Possibilities and Limitations

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The main task of ultrastructural telepathology is to deliver adequate electron microscopy (EM) findings in the shortest amount of time to establish or support pathological diagnoses from a remote location. As in light-pathology, the consultation of experts is essential for complex EM cases (tumours, renal, skin, muscle, nervous system, ciliar and storage diseases; rapid viral diagnosis – including bioterror scenario), and original specimens need to be examined directly instead of interpreting pre-selected images.

We used a remote EM diagnostic system based on the LEO912AB TEM equipped with a CCD-camera, and controlled by the "analySIS" software from a server and linked via Internet to locations throughout Europe. The standard software (EsiVision, Soft Imaging System, Germany) was expanded with a dedicated "TelePresence-Server-Module" that handles the communication between the EM and the remote expert. At the "client site" (experts located in Berlin, Koblenz, Zurich, and Innsbruck) desktop-PC or a notebook running the same software expanded with a "Client-Module" were applied.

Ultrathin sections of selected diseases and negative-stained specimens containing virus particles were examined remotely live via Internet. The remote EM control included: stage navigation and search for area of interest at low magnification and resolution, selection of adequate magnification (18 - 400 000x), focus adjustment, beam brightness and exposure time control, and the local image documentation at full resolution.

Remote EM examination of pathological samples has been established by combining the full digital and highly automated EM with digital image acquisition, telepresence microscopy techniques, and rapid advances in the Internet technology.