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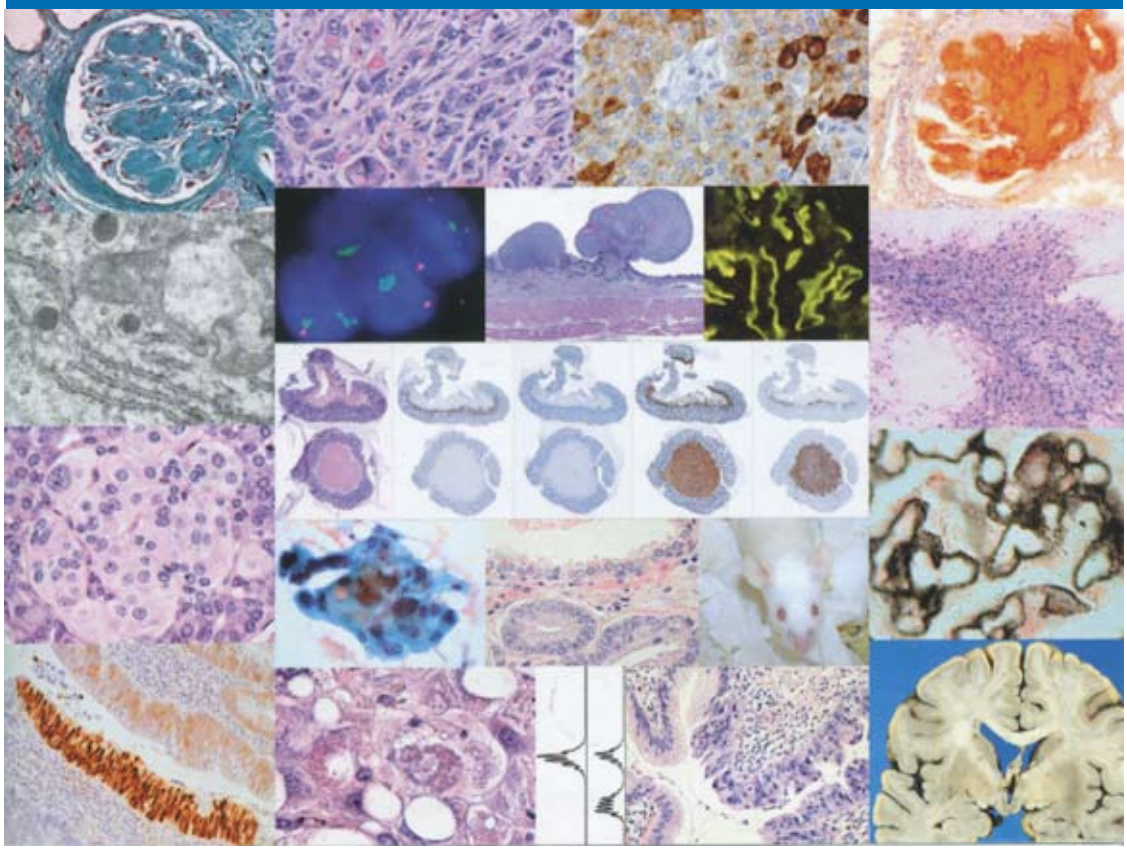
BELGIAN WEEK OF PATHOLOGY

BELGIAN WEEK OF PATHOLOGY 2010

April 21-24, 2010
Het Pand – Ghent



www.bwpath.be

WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

cmmi

excellence in preclinical imaging

&

Have made the Slide Seminar available on the website:

www.bwpath.be

WELCOME

Dear Colleagues and Friends,

The **second edition of the Belgian Week of Pathology** will be hosted in Ghent, Belgium, from April 21 to 24, 2010. Boosted by the success of the first BWP we decided to renew our commitment. However, this second edition has some news in store for you. We hope you will enjoy it.

The BWP was born in 2008 from the desire of various societies to promote high quality diagnostic practice in human and veterinary pathology. The BWP includes eight societies and groups: SBAP/BVPA, SBCC/BVCC, GBS, Belgian Legal Medicine Society, Belgian Club of Digestive Pathology, BBTG, Belgian Dermatopathology and Veterinary pathology.

The Organizing Committee has prepared an interesting and informative scientific programme, intending to gather a wide international faculty of experts in several fields of Pathology. Our goal is to respond to your current professional concerns. In recent years, our speciality has mutated from pure morphology to what I call "biomorphology". As a consequence we have to extend our technical and scientific knowledge in order to include new techniques in our daily practice diagnosis. The selected topics have integrated this philosophy.

The congress will have separate sections on lung pathology, lymphomas, uterine cervix and breast pathology, digestive pathology, veterinary pathology: bacterial and viral zoonoses, brain tumors, dermatopathology, legal medicine and ethics. We will welcome around thirty American and European colleagues. It is a great honor for us that Pr. zur Hausen agreed to give the opening lecture. He was Nobel Prize laureate for the discovery of HPV as a cause for cervical cancer. We also had a pleasure of receiving about forty free papers of great interest. The best oral presentation will be awarded a prize offered by the Yvonne Boël Foundation and the two best posters will be rewarded by Merck. Slide seminars have been improved considerably by using virtual microscopy with the help of Hamamatsu and DIAPATH–CMMI.

We deeply thank our partners in the industry for their important renewed support. Seven satellite symposia will be organised by several companies around topics of the utmost interest in daily practice, molecular testing and virtual pathology.

Sessions will take place in the magnificent Pand, near the center of the city, within a walking distance of several hotels, museums and shopping centers. Ghent, a beautiful city, enjoys a favourable geographic and historical position and offers a pleasant environment for a successful scientific congregation. We will be happy to welcome you for dinner on Friday night in the warm atmosphere of an elegant private club at the Falligan Hotel.

On behalf of the scientific and organizing committee I wish you an excellent BWP 2010.

Isabelle Salmon,

BWP 2010 President

WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

La microscopie virtuelle…
2 fois plus rapide !

NanoZoomer 2.0 : une nouvelle génération, 2 fois plus rapide.

NanoZoomer

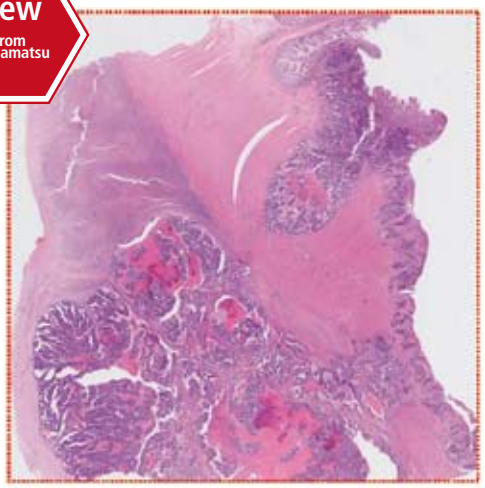
Approx. 3 minutes

En mode de scan 20X, zone de 20 x 20 mm

Doublez
vos vitesses de scan !

NanoZoomer 2.0

New from Hamamatsu



Approx. 1 minute 40 secondes

- **Vitesse de scan améliorée au 40X, idéal pour des scans en routine**
  Le NanoZoomer 2.0 réduit le temps de scan d'environ 12 min à 4.5 min seulement.

- **Possibilité de conversion du NanoZoomer au NanoZoomer 2.0**
  Nécessite l'achat du matériel prévu et la mise à jour par notre équipe.

- **NanoZoomer 2.0 HT**
  Scan automatique jusqu'à 210 lames.

- **NanoZoomer 2.0 RS**
  Scan de lames standards (X6) ou de grandes lames (X2).

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INDEX




GENERAL INFORMATION

Accreditation

Certificates for accreditation will be provided at the end of each half day session (4 CP units) to the registered delegates who have attended the sessions.
(Typr-Rubriek 3 – CP 24 – Organizer: BWP 2757)

Language

The language of the congress is English (British spelling) for abstracts, slides and announcements.

Abstracts

Authors were invited to send abstracts until February 28, 2010. 36 abstracts were submitted to the Selection Committee: 11 were selected as oral presentations and will be presented on Thursday during the session on Cervical Pathology and the Free Paper Session; 25 Posters will be presented during the Poster Session on Friday from 12:00 to 13:00.

Prizes will be awarded on Friday afternoon at the end of the scientific program:

- The Boël Foundation will award the Best Oral Presentation with 2.000€
- Merck will award 2 Posters with 500€ each:
  - 1 for the Best Original Research
  - 1 for the Best Clinical Case

Venue

Het Pand – Onderberg, 1 – 9000 Ghent

Conference rooms are on the first and the second floor. The exhibition, poster area and registration are on the groundfloor.

Parking

Several parkings are located close the congress venue:

- Parking 7 – St Michiels: across the street from the congress venue
- Ramen Parking: in front of the cathedral
- Parking Kouter

Hotels

NH Hotel Ghent Belfort – Tel: +32 (0)9 233 33 31 / Fax: +32 (0)9 233 11 02
Marriott Ghent – Tel: +32 (0)9 233 33 93 / Fax: +32 (0)9 233 33 94

Event Coordinator

Anne-France De Meyer – 102, Av.Carsoel – 1180 Brussels – Belgium
Tel : +32 2 375 36 26 / Fax : +32 2 375 47 84 / E-mail : anne.france.de.meyer@skynet.be

Ghent Tourism Office

Botermarkt, 17A – 9000 Ghent
Tel : +32 9 266 52 32

STEERING COMMITTEE

Scientific Societies:

Belgian Society of Clinical Cytology (BVKC – SBCC)
Belgian Society of Pathology (BVPA – SBAP)
BelgianClub of Digestive Pathology
Belgian Association of Pathologists (GBS – VBS)
Belgian Royal Society of Forensic Medicine (KBGGG – SRMLB)
Belgian Working Group on Animal Pathology
Belgian Group of Brain Tumors
Belgian Group of Neuropathology
Belgian Club of Dermatopathology

President :	I. Salmon
Vice-President :	L. de Leval
Honorary President :	J.P. Cosyns
Treasurer :	B. Weynand
Secretaries :	C. Cuvelier P. Demetter
Congress coordinator :	A. Jouret-Mourin
Executive committee member :	J.P. Bogers

Members :

J. André
F. Bonbled
C. Bourgain
K. Cokelaere
P. Cras
P. Delvenne
C. Godfraind
M. Heimann
A. Hoorens
M. Kevers
M. Laporte
E. Marbaix
A. Michotte
S. Roels
T. Roskams
C. Sempoux
L. Thienpont
C. Van den Broecke
D. Van Varenbergh
F. Willocx

Your Partner in Molecular Diagnostics

Satellite Symposia:

22 April, 14:30 – 15:30, Infirmary Room
'Prepare for the Future: QIAGEN's solutions for cervical cancer screening'

Dr. Geraldine Roeder, Women's Health Specialist from UK, introduces you in the most up to date possibilities of cervical cancer screening. From evidence to implementation – learn about the clinical use of HPV DNA testing today and tomorrow.

24 April, 8:15 – 9:00, Infirmary Room
'QIAGEN's solutions for Molecular Pathology'

Coffee & Croissants

Dr. Peter Van Hauwe, QIAGEN Account Manager Molecular Diagnostics from Belgium, will give you an overview of the latest developments within QIAGEN. The application of molecular technologies in pathology is increasing steadily. During this symposium you will be able to learn more about the use of techniques like real-time PCR, pyrosequencing and hybrid capture – with specific focus of the use in the field of pathology.

BELGIAN FACULTY

J. André
E. Barale -Thomas
L. Bogaert
J.P. Bogers
F. Bonbled
G. Boulet
C. Bourgain
K. Cokelaere
J.P. Cosyns
P. Cras
C. Cuvelier
A.-L. Delezoide
L. de Leval
P. Delvenne
P. Demetter
K. De Raedt
R. Ducatelle
D. Faverly
C. Galant
C. Godfraind
E. Goossens
M. Govaerts
M. Heimann
P. Heyman
A. Hoorens
A. Jouret-Mourin
A. Jouvet
M. Kevers
M. Laporte
B. Maillet
E. Marbaix
J. Mast
A. Michotte
P. Moerman
M. Remmelink
S. Roels
T. Roskams
X. Sagaert
I. Salmon
C. Sempoux
I. Theate
L. Thienpont
C. Van den Broecke
D. Van Varenbergh
B. Weynand
F. Willocx

INTERNATIONAL FACULTY

R. Barnhill	Paris - France
P. Bioulac-Sage	Bordeaux - France
F. Bosman	Geneva - Switzerland
P. Burger	New-York - USA
J. Cuzick	London - United Kingdom
D. Di Bonito / A. Romano	Trieste - Italy
C. Favrot	Basel - Switzerland
D. Figarella-Branger	Marseille - France
J.-F. Fléjou	Paris - France
D. Flieder	Philadelphia - USA
R. Hasserjian	Boston - USA
H. Kerl	Graz - Austria
K. Kerr	London - United Kingdom
T. Kirchner	Munich - Germany
B. Kubat	The Hague - Netherlands
R. Küppers	Essen - Germany
S. Lantuejoul	Lyon - France
J. Mack	Hershey - USA
E. Mark	Boston - USA
J. Monsonego	Paris - France
H. Popper	Frankfurt - Germany
L. Quintanilla-Fend	Tuebingen - Germany
V. Schneider	Freiburg - Germany
W. Squier	London - United Kingdom
A. van Driel	Amsterdam - Netherlands
R. van Rijn	Utrecht - Netherlands
P. Vielh	Paris - France
P. Wesseling	Nijmegen - Netherlands
M. von Knebel Doeberitz	Frankfurt - Germany
H. zur Hausen	Frankfurt - Germany

SATELITTE SYMPOSIA

Wednesday April 21	**14:30 – 15:30** **Rector Vermeylen Room**	**SAKURA** 14:30-14:50 14:50-15:10 15:10-15:30	**«The way to a highly efficient Histo-Pathology Laboratory»** Speaker: Prof. Dr. Axel zur Hausen (Germany, Freiburg); Prof. Dr. Axel zur Hausen will focus on the following • How to organize a lab in being more efficient • Advantages in being more efficient • The core of being more efficient
Thursday April 22	**13:00-14:30** **Rector Vermeylen Room**	**HOLOGIC** 13:00-13:45 13:45-14:30	**Cervista HR HPV method** **Cervista ™ Third Wave Invader method for high-risk HPV detection: a serious Alternative for the Hybrid Capture II method?** Speaker: P.A. Jaarsma
	14:30 -15:30 **Infirmary Room**	**QIAGEN** 14:30-15:30	**Prepare for the Future: QIAGEN's solutions for Cervical Screening".** speaker: Geraldine Roeder, Crawley UK (QIAGEN)
	16:30-18:00 **Rector Vermeylen Room**	**ROCHE** 16:30-16:45 16:45-17:00 17:00-17:15 17:15-17:30 17:30-18:00 18:00-18:30	**HER-2 testing** Chairpersons: M. Kockx, D. Faverly, E. de Azambuja Her-2 testing: everything starts here E. de Azambuja Belgian guidelines for Her-2 testing in breast cancer – anno 2010 C. Colpaert Results of a multi-center retrospective study to determine the pourcentage of false negative in patients scored as IHC 0 and 1+ D. Larsimont Belgian register for IHC 1+ patients with FISH result P. Pauwels Her-2 testing in gastric cancer J. Rüschoff Questions and answers

SATELITTE SYMPOSIA

Friday April 23	**12:00-13:00** **Rector Vermeylen Room**	**LILLY**	Chairperson: **B. Weynand**
		12:00-12:30	The predictive and/or prognostic role of TS in thoracic tumours K. Kerr
		12:30-13:00	Consensus guidelines in the diagnostic pathology of NSCLC B. Weynand
	14 :00-15 :00 **Rector Vermeylen Room**	**HAMAMATSU**	**Role of Adipose Tissue : Histopathological Analysis using Virtual Slides (HAMAMATSU/GSK)** Speaker : Q.-N. Trinh-Xuan, Paris-France
		14:00-14:30	Virtual Microscopy Introduction
		14:30-15:00	Application on Adipose Tissue
	17:30-19:00 **Rector Vermeylen Room**	**ROCHE**	**Lung cancer: NSCLC and personalised healthcare: myth or reality?** Chairpersons: J. De Grève, M. Praet, V. Ninane
		17:30-17:40	Introduction V. Ninane
		17:40-18:00	Biomarkers in NSCLC: prognostic and predictive factors – status of today M. van de Vijver
		18:00-18:20	Clinical impact of targeted therapy on EGFR mutation positive and wild-type E. Smit
		18:20-18:40	EGFR testing - future evolution and need of common recommendations P. Demetter
		18:40-18:55	Questions and answers J. De Grève and round table discussion
		18:55-19:00	Closing and remarks M. Praet
		19:00	Reception
Saturday April 24	**8 :15 -9 :00** **Infirmary Room**	**QIAGEN + Breakfast**	**QIAGEN's solutions for molecular pathology** speaker: P. Van Hauwe, Antwerp Belgium (QIAGEN)

PROGRAM OVERVIEW

WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

		REFTER ROOM Ground floor	RECTOR VERMEYLEN First floor	INFIRMARY Second floor	RECTOR BLANQUAERT Third floor
WEDNESDAY April 21	14:00	Opening : I.Salmon	Satellite Symposium SAKURA		
	14:00 - 16:00	Hodgkin lymphoma and its mimics	Ethics		
	16:00 - 16:30	Coffee Break "Ehibition Area"			
	16:30 - 18:00	Hodgkin lymphoma and its mimics	Ethics		
	18:00 - 19:00	Opening Lecture: Nobel Prize - H. zur Hausen			
THURSDAY April 22	8:00 - 9:50		Current issues in uterine cervix pathology	Current issues in brain tumors	Veterinary Pathology
	9:50 - 10:10	Coffee Break «Exhibition Area»			
	10:10 - 13:00		Current issues in uterine cervix pathology	Current issues in brain tumors	Veterinary Pathology
	LUNCH 13:00 - 14:00	"Exhibition Area"			
	13:00 - 14:30		Satellite Symposium HOLOGIC		
	14:00 - 16:00		Current issues in breast pathology	Satellite Symposium QIAGEN	Viral zoonosis and use of laboratory animals
	16:00 - 16:30	Coffee Break «Exhibition Area»			
	16:30 - 18:30		Satellite Symposium ROCHE	Free paper session	Viral zoonosis and use of laboratory animals
FRIADAY APRIL 23	8:30 - 10:30		Neoplastic Pathology and Cytology of Lung		Legal Medicine
	10:30 - 11:00	Coffee Break «Exhibition Area»	SBAP General Assembly		
	11:00 - 13:00		Neoplastic Pathology and Cytology of Lung		Legal Medicine
	12:00 - 13:00	Poster Session	Satellite Symposium LILLY		
	13:00 - 13:30	«Exhibition Area»			
	13:30 - 15:30		Neoplastic Pathology and Cytology of Lung	Satellite Symposium HAMAMATSU	Pathology of Placenta
	15:30 - 16:00	Coffee Break «Exhibition Area»			Pathology Club General Assembly
	15:30 - 17:30		Neoplastic Pathology and Cytology of Lung		Update on Digestive Pathology
			Awards: - Best Oral Presentation - 2 Best Posters		
	17:30 - 19:00		Satellite Symposium ROCHE		
SATURDAY April 24	8 :15 – 9:00			Satellite Symposium QIAGEN	
	9:00 - 10:30		Cervico-vaginal Cytology	Basic molecular Pathology	Dermatopathology
	10:30 - 11:00	Coffee Break "Exhibition Area"	SBCC General Assembly		
	11:00 - 12:15		Cervico-vaginal Cytology	Basic molecular Pathology	Dermatopathology
	12:15 - 12:30		Closing: I. Salmon		

Summary of the Independent ASCO/CAP HER2 Testing Guidelines

A complete copy of the guidelines may be found on the ASCO and CAP websites: www.asco.org and www.cap.org

Tissue Handling Standardisation

- 10% neutral buffered formalin–optimal fixation times: 6 to 48 hours and should be documented on the pathology report.
- Alternative fixative (e.g. PREFER, Alcoholic Formalin, etc.) must be validated against the results of the identical specimen fixed in 10% NBF.
- Cut sections stored longer than 6 weeks are not optimal for testing and should be re-cut.

Method Validation Requirements

- Testing must be initially validated using a 25-100 formalin-fixed samples using standard operating procedures.
  - Parallel testing by an alternative method within your lab (e.g. FISH, SISH) is acceptable.
  - Parallel testing by an identical method in another lab with the same validated assay is also acceptable.
- Validation should be performed twice a year (external proficiency exam testing meets this requirement).
- Laboratory/Lab Director is ultimately responsible for the validation.
- Assay procedures must be standardised.
  - Any deviations from the standardised procedure must be validated.
  - Any changes from procedure must be documented on the report.
- Optimal performance is easily obtained using automated platforms.
- Personnel must have their competency assessed at regular intervals.

Standardised Control Materials

- Cell line controls or tumour blocks with well defined negative, equivocal and positive expression need to be used. If controls do not show usual results, the assay must be repeated rather than interpreted.
- Controls need to be used by the laboratory with each run of tests.

Image Analysis

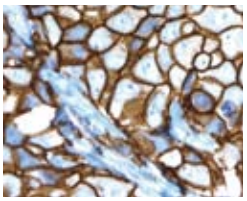
- Image analysis is an effective tool for achieving consistent interpretation of HER2. Pathologists must confirm the image result.
- Image analysis must be validated before implementation.
- Image analysis instrument must be calibrated regularly.
- Quantitative image analysis is encouraged for cases with weak membrane staining (1 to 2+) to improve consistency of interpretation.

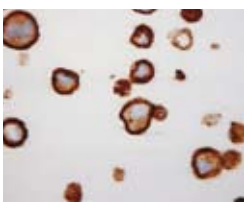
Reporting

- Only evaluate invasive breast cancer or the invasive component of the breast cancer
- More than 30% of the tumour must show circumferential membrane staining for positive results

Positive Score: IHC 3+	FISH >6.0 gene copy	FISH HER2/CEP17 ratio >2.2
Equivocal Score: IHC 2+	FISH 4.0-6.0 gene copy	FISH HER2/CEP17 ratio 1.8-2.2
Negative Score: IHC 0 or 1+	FISH <4.0 gene copy	FISH HER2/CEP17 ratio <1.8

- If cytoplasmic staining obscures membrane staining, repeat assay or do FISH test.
- For an IHC equivocal result, the specimen must be retested with a validated assay for gene amplification.
- Reject if lobules or normal ducts show obvious staining.
- Report should include:
  - Specimen site and type and specimen fixation time.
  - Antibody clone/vendor/method used and if FDA approved (if modified, this should be stated and a statement that the lab takes responsibility for test performance)
  - Image analysis results methods (if used)
  - Controls : protein expression in the controls
  - Adequacy of sample for evaluation
  - Results % invasive tumour cell showing complete staining.
  - Uniformity (present or absent), dark circumferential staining (present or absent).





Proficiency Assessment

- Participation in an external proficiency exam with at least two testing events/year.
  - Satisfactory performance requires at least 90% correct responses on graded challenges for either test.
  - Unsatisfactory performance will require the laboratory to respond according to the requirements of the accreditation agency programme.

Assisting with your efforts to be ASCO/CAP Compliant

Fully Integrated Ventana Breast Panel Biomarkers

HER-2/neu (4B5)	PATHWAY anti-HER-2/neu (clone 4B5) Rabbit Monoclonal Primary Antibody is intended for laboratory use for the semi-quantitative detection of HER-2 antigen in sections of formalin-fixed, paraffin-embedded normal and neoplastic tissue. It is indicated as an aid in the assessment of breast cancer patients for whom Herceptin® treatment is considered.
ER (SP1)	CONFIRM anti-Estrogen Receptor (SP1) Primary Antibody is a rabbit monoclonal antibody that is intended for laboratory use for the qualitative detection of estrogen receptor (ER) antigen in sections of formalin-fixed, paraffin-embedded tissue. CONFIRM anti-ER (SP1) is directed against an epitope present on human ER protein located in the nucleus of ER positive normal and neoplastic cells.
PR (1E2)	CONFIRM anti-Progesterone Receptor (1E2) Primary Antibody is intended for laboratory use for the qualitative detection of progesterone receptor (PR) antigen in sections of formalin-fixed, paraffin-embedded tissue on a Ventana automated slide-stainer. CONFIRM anti-PR (1E2) recognises the A and B forms of human progesterone receptor and is indicated as an aid in the management, prognosis, and prediction of therapy outcome of breast cancer.
Ki-67 (30-9)	CONFIRM anti-Ki-67 (30-9) Primary Antibody is directed against C-terminal portion of Ki-67 antigen and is intended for use to identify proliferating cells by light microscopy in sections of formalin-fixed, paraffin-embedded tissue.
HER2 4-in-1 Control Slides	PATHWAY HER2 4-in-1 Control Slides consist of formalin-fixed, paraffin-embedded, cultured human breast cell lines and are intended to be used as an assayed, semi-quantitative quality control in conjunction with PATHWAY HER2 (4B5) primary antibody. They are used for monitoring the performance of the immunohistochemical anti-c-erbB-2/HER-2 staining process on a Ventana automated slide-stainer.
HER*2* SISH / Dual Colour ISH	INFORM HER2 DNA Probe is designed to quantitatively detect amplification of the HER2 gene via chromogenic silver in situ hybridisation (SISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens following staining on Ventana automated slide-staining instruments using light microscopy. Results from the INFORM HER2 DNA Probe are intended for use as an adjunct to existing clinical and pathologic information currently used for estimating prognosis in patients with invasive breast cancer.

CE

Rabbit Monoclonals

ER (SP1)
#790-4324
05278406001

PR (1E2)
#790-2223
05277990001

Ki-67 (30-9)
#790-4286
05278384001

HER-2/*neu* (4B5)
#790-2991
05278368001

Probes

HER2 SISH
#780-4332
05273439001

Control

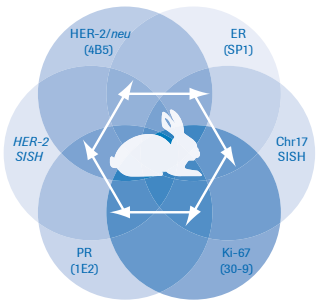
HER-2 4-in-1 Control Slides *#781-2991*
05273510001

Slide preparation automation with BENCHMARK XT
N750-BMKXT-FS 05285437001

Image Analysis and integrated patient report from VIAS
VIAS-799-CLOCK-220 05286255001

Connection LIS
Ventana Lab Manager *#2105200* 05259002001
Ventana Interface Point *#1364000* 0524786100

Ventana part numbers in black, Roche item number in blue



Breast Panel System

Patient Centred Pathology

WORKFLOW | DIAGNOSTICS

E601 1208B

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EC REP
Roche Diagnostics GmbH
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68305 Mannheim
Germany
Tel. +49 (0)621 7590

www.ventanamed.com

Roche

WEDNESDAY 21 AFTERNOON

ROOM: Refter Room

14:00-14:05 **Welcome**
I. Salmon

Hodgkin lymphoma and its mimics
Chairpersons : *L. de Leval, I. Theate*

14:05-14:40 Pathobiology of Hodgkin lymphomas
R. Küppers
14:40-15:20 Pathology of Hodgkin lymphomas
R. Hasserjian
15:25-16:00 The borders between Hodgkin and non-Hodgkin lymphomas
L. Quintanilla-Fend
16:00-16:30 Coffee break
16:30-18:00 Teaching cases
R. Hasserjian, L. Quintanilla-Fend, L. de Leval, I. Theate

ROOM: Rector Vermeylen

14:30-15:30 **Satellite symposium: SAKURA**
"The way to a highly efficient Histo-Pathology Laboratory"

ROOM: Rector Vermeylen

Ethics:
Future of pathology in view of expected manpower and new regulations in Europe
Chairpersons: *E. Marbaix, K. Cokelaere*

15:30-16:00 How does European Union legislation influence the practice of the pathologist?
B. Maillet
16:00-16:30 Coffee break
16:30-17:00 Life long learning in pathology
F. Bosman
17:00-17:30 The evolution of the pathologist and of pathology practice
J.-F. Fléjou
17:30-18:00 Expectations for manpower in pathology practice
C. Cuvelier

ROOM: Refter Room

18:10-19:00 Opening lecture - Chairperson: J.-P. Bogers

Speaker: Nobel Prize, H. zur Hausen
Infections causing human cancers

THURSDAY 22 MORNING

THURSDAY

ROOM: Rector Vermeylen

Current issues in uterine cervix pathology

Chairpersons: *J.-P. Bogers, Ph. Delvenne*

08:00-08:45		Screening from the side of the gynaecologist *J. Monsonego*
08:45-09:30		Screening from the side of the epidemiologist *J. Cuzick*
09:30	**O 01**	Selected free paper: Nucleic acid-sequence based amplification assay for HPV mRNA detection and typing: evidence for DNA amplification *I. Micalessi, G. Boulet, I. Benoy, C. Depuydt, M. Ieven, P. Van Damme, J.-P. Bogers*
09:50-10:10		Coffee-break
10:10-10:55		Role of cytology in screening *V. Schneider*
10:55-11:40		Role of adjunctive testing / molecular markers in screening *M. von Knebel Doeberitz*
11:40	**O 02**	Selected free paper: What's new about HPV and HNSCC? *D. Bafort, Y. Guiot, S. Schmitz, J.P. Machiels, H. Reychler, V. Gregoire, B. Weynand/ UCL St-Luc*
12:00-13:00		Keynote lecture - Chairperson: Ph. Delvenne **Speaker: Nobel Prize, H. zur Hausen** "Papillomaviruses and human cancers: basic studies and clinical application".

THURSDAY 22 MORNING

ROOM: Rector Blanquaert

Veterinary pathology - Specific pathology and Bacterial zoonosis
Chairpersons: *S. Roels, M. Heimann*

08:30-09:10	Electron microscopy: past and future *J. Mast*
09:10-09:50	A new chicken model for human inflammatory bowel disease *R. Ducatelle*
09:50-10:10	Coffee-break
10:30-11:15	TBC infections in man and animals *M. Govaerts*
11:15-12:00	Leptospirosis in man and animal *E. Goossens*

ROOM: Infirmary

Current issues in brain tumours
Chairpersons: *I. Salmon, C. Godfraind*

08:30-09:10	Neural stem cells and gliomagenesis *D. Figarella-Branger*
09:10-09:50	Angiogenesis / anti-angiogenic therapy of brain tumours *P. Wesseling*
09:50-10:10	Coffee-break
10:10-10:50	Classification of pediatric CNS tumours *P. Burger*
10:50-12:00	Slide seminar pediatric CNS tumours Speakers: *D. Figarella-Branger, C. Godfraind, P. Burger, A. Jouvet*
13:00-14:00	Lunch

THURSDAY 22 AFTERNOON

ROOM: Rector Vermeylen

13:00-14:30 **Satellite Symposium : Hologic Benelux**

"Cervista HR HPV method"

ROOM: Infirmary

13:00-14:30 **Satellite Symposium QIAGEN**

Prepare for the Future: QIAGEN's solutions for Cervical Screening".

ROOM: Infirmary

Free paper session

Chairpersons: *J.-P. Cosyns, P. Demetter*

16.30 **O 03** Combined analysis of HPV-DNA, p16, p21 and p53 to predict prognosis in stage IV hypopharyngeal carcinomas
P. Ernoux-Neufcoeur, M. Arafa, C. Decaestecker, D. Chevalier, X. Leroy, M. Herfs, J. Somja, C. Depuydt, P. Delvenne, S. Saussez

16.42 **O 04** Indoleamine 2,3-dioxygenase expression at the tumor invasion front is an independent negative prognostic factor in pT1-4N1Mx staged colorectal cancer
L. Ferdinande, C. Decaestecker, A. Mathieu, L. Verset, X. Moles-Lopez, A.-M. Negulescu, T. Van Maerken, I. Salmon, C. Cuvelier, P. Demetter

16.54 **O 05** Ki-67 hot-spots detection on glioblastoma tissue sections
X. Moles Lopez, O. Debeir, C. Maris, X. Catteau, I. Roland, I. Salmon, C. Decaestecker

17.06 **O 06** Prevalence of metaplasia in salivary gland pleomorphic adenoma
J.-C. Tille, H. Reychler, M. Hamoir, S. Schmitz, B. Weynand

17.18 **O 07** hERα36, a new variant of ERα: expression and potential biological relevance in breast cancer
V. Pelekanou, I. Laios, E. Castanas, G. Leclercq, D. Larsimont

17.30 **O 08** A taxonomy of epithelial human cancer and their metastases
O. Gevaert, A. Daemen, B. De Moor, L. Libbrecht

17.42 **O 09** Galectin-1 and -3 have synergistic effects on angiogenesis
N. D'Haene, C. Maris, S. Sauvage, C. Decaestecker, I. Salmon

17.54 **O 10** Secondary tumors within the large bowel: origin and frequency
B. Leonard, D. Leonard, C. Remue, R. Detry, A. Kartheuser, C. Sempoux, A. Jouret-Mourin

18.06 **O 11** CA9 expression in multilocular cystic renal cell carcinoma
F. Sciotto, M. Gremaud, M.-F. Pelte, J.-C. Tille

THURSDAY 22 AFTERNOON

ROOM: Rector Blanquaert

Viral zoonosis and the use of laboratory animals

Chairpersons: *S. Roels, M. Heimann*

14:00-15:00	New insight in papillomavirus-induced skin and mucous membranes carcinogenesis *C. Favrot*
15:00-15:20	"Bowens", "Bowenoid" … the histopathological clues *M. Heimann*
15:20-16:00	Papillomavirus in equine skin lesions *L. Bogaert*
16:00-16:30	Coffee-break
16:30-17:00	TBE in man and animal *S. Roels*
17:00-17:30	Hantaviruses in man and animal *P. Heyman*
17:30-18:00	Animal experiments: animal protection and ethics *E. Barale-Thomas*

ROOM: Rector Vermeylen

Current issues in breast pathology

Chairpersons: *E. Marbaix, C. Galant*

14:00-14:45	Pathologist input in a one stop clinic for breast lesions *P. Vielh*
14:45-15:30	Pathology of breast carcinoma after neoadjuvant chemotherapy *D. Faverly*
15:30-16:00	Slide seminar
16:00-16:30	Coffee-break
16:30-18:30	**Satellite Symposium : ROCHE** **"HER-2 testing"**

FRIDAY 23 MORNING

ROOM: Rector Blanquaert

Legal medicine

Chairpersons: *D. Van Varenbergh, F. Bonbled*

08:30-09:10	Anatomy and development of the meninges: implications for subdural collections and CSF circulation *J. Mack*
09:10-09:50	The neuropathology of infant subdural haemorrhage *W. Squier*
09:50-10:30	Neuroradiologic findings in young children with subdural haemorrhage *R. van Rijn*
10:30-11:00	Coffee-break
11:00-11:40	Early and late patterns of diffuse traumatic brain injury in young children. *B. Kubat*

ROOM: Rector Vermeylen

Neoplastic pathology and cytology of lung

Chairpersons: *B. Weynand, M. Remmelink*

09:00-09:45	Lung tumours and in particular preneoplastic lesions *K. Kerr*
09:45-10:30	The new TNM classification and its implications on macro- and microscopic analysis of lung cancer specimens *D. Flieder*
10:30-11:00	Coffee-break General Assembly Belgische Vereniging voor Pathologische Anatomie-Société Belge d'Anatomie Pathologique
11:00-11:30	Neuroendocrine tumours of the lung *S. Lantuejoul*
11:30-12:00	Molecular markers in lung carcinoma *S. Lantuejoul*
12:00-13:00	**Satellite Symposium : LILLY** **"The predictive and/or prognostic role of TS in thoracic tumours"** **"Consensus guidelines in the diagnostic pathology of NSCLC"**
12:00-14:00	Poster session – Chairpersons: *C. Cuvelier, L. Thienpont*
13:00-13.30	Lunch

FRIDAY 23 AFTERNOON

ROOM: Infirmary

14:00-15:00 **Satellite Symposium: HAMAMATSU**
"Role of Adipose Tissue : Histopathological Analysis using Virtual Slides"

ROOM: Rector Vermeylen

Neoplastic pathology and cytology of pleura
Chairpersons: *B. Weynand, M. Remmelink*

13:30-14:20 Pathological aspects of mesothelioma
E. Mark

14:20-15:00 Cytology of pleural effusions
A. Romano and L. Di Bonito

15:00-15:30 Molecular markers in malignant pleural pathology
H. Popper

15:30-16:00 Coffee-break

16:00-17:30 Slide seminar Belgian Mesothelioma Registry

ROOM: Rector Blanquaert

Pathology of placenta
Chairpersons: *C. Bourgain, P. Moerman*

14:00-14:30 Development and structure of the placenta
A.-L. Delezoide

14:30-15:00 Placental infection and inflammation
C. Bourgain

15:00-15:30 New insights in gestational trophoblastic disease
P. Moerman

Update on digestive pathology
Chairpersons: *A. Jouret-Mourin, A. Hoorens*

15:30-16:00 General Assembly Belgische Club voor Digestieve Pathologie-Club Belge de Pathologie Digestive

16:00-16:20 New classification of hepatocellular adenomas
P. Bioulac-Sage

16:20-16:40 New classification of primary liver carcinomas
T. Roskams

16:40-17:00 Lymphoid hyperplasia and MALT lymphoma
I. Theate

17:00-17:30 Serrated polyps and the serrated pathway
T. Kirchner

17:30 **AWARDS:** ***Best Oral Presentation: Boël Prize***
Best Posters: 2 MERCK Prizes

ROOM: Infirmary

17:30-19:00 **Satellite Symposium: ROCHE**
"NSCLC and personalised healthcare: myth or reality?"
Followed by a drink

SATURDAY 24 MORNING

ROOM: Infirmary

8:15 -9 :00 **Satellite Symposium QIAGEN**
"QIAGEN's solutions for molecular pathology"
With Breakfast

ROOM: Rector Vermeylen

Cervico-vaginal cytology
Chairpersons: *L. Thienpont, F. Willocx*

09:00-10:30	High-grade intraepithelial squamous lesions *A. Romano and L. Di Bonito*
10:30-11:00	Coffee-break
11:00-11.15	General Assembly Belgische Vereniging voor Klinische Cytologie-Société Belge de Cytologie Clinique
11:15-11:45	Glandular endocervical lesions *A. van Driel*
11:45-12:15	Slide seminar

ROOM: Rector Blanquaert

Dermatopathology
Chairpersons: *M. Laporte, J. André*

09:00-09:45	Slide seminar
09:45-10:30	Spitz tumours *R. Barnhill*
10:30-11:00	Coffee-break
11:00-11:45	Malignant melanoma *H. Kerl*
11:45-12:00	Discussion

ROOM: Infirmary

Basics in molecular pathology
Chairpersons: *J.-P. Bogers, T. Roskams*

09:00-09:45	Molecular pathology: current clinical practise and what to expect in the future *X. Sagaert*
09:45-10.30	Background and practical guidelines for Her-2/Neu FISH *K. De Raedt*
10.30-11:00	Coffee-break
11:00-12:00	Molecular markers in cervical cancer screening: an overview of tools and techniques *G. Boulet*

ROOM: Infirmary

12:15-12:25 **Closing remarks**
I. Salmon

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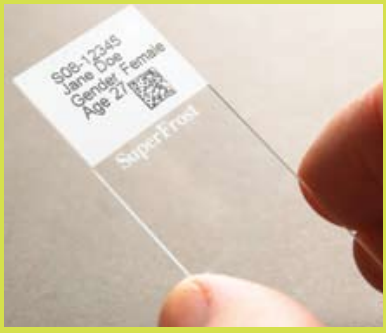

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April, 21 afternoon

April, 22 to 24

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C 2	SAKURA
C 3	KLINIPATH
C 4	BD
C 5	SARSTEDT
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C 11	LEICA
C 12	GEN-PROBE
C 13	INNOGENETICS
C 14	MEDINVENTS
C 15	QIAGEN
C 20	ASTRAZENECA
C 21	TRIBVN
C 22	MENARINI
C 23	PROSAN
C 24	LILLY
C 25	MLS
C 26	BMD
D	DIAGOMICS
E	MERCK

CONGRESS DINNER

Venue:

Hotel Falligan,
Société Royale Littéraire, Kouter, 172
9000 Ghent

April 23, 2010

Aperitive: 8.00 pm

Dinner: 9.00 pm

Enjoy a nice evening in an elegant private club!

Invitation card will be requested at the entrance

Dress: wearing a tie is absolutely mandatory for men

INVITED LECTURES

INVITED LECTURES LIST

INVITED LECTURES ABSTRACTS

L01

Protection of the laboratory animals and ethical committees on animal experimentation

E. Barale-Thomas, Johnson & Johnson Pharmaceutical Research & Development, Beerse, Belgium

Protecting the laboratory animal: why?

Because as we use the animal (right), then we must treat it well (duty)

- Take care of it if necessary
- Act correctly (technical procedures, pain management)
- The team "right / duty" is related to other notions like "allowed / forbidden", "good / evil". These notions are typical of a reflection on ethics and an implementation of legal measures
- The legal measures are prepared after the ethical reflection

But also because such a protection has also positive consequences on the study results

- The link between study quality, animal well-being and ethical conditions in the laboratory has been shown in several scientific publications

Focus: utilitarianism and deontology

Utilitarianism: doctrine which puts what is useful the center of all values, in the fields of knowledge as of action. The main principle of this doctrine is the quantification of the pleasures of individuals, which should lead to the maximization of happiness for the greatest number among the population

- A decision is morally right if the sum of good it brings is greater than the sum of evil (good is explicitly identified with pleasure and happiness)
- Therefore animal experimentation is acceptable, because it produces more good (knowledge, drugs…) than evil (the animal suffering generated)

Ethics / deontology: theory or study of moral duties (by extension, moral rules that govern the exercise of an occupation or the social relations of its members, e.g. the code of ethics for physicians, veterinarians, lawyers)

- A theory is deontological if and only if certain actions are deemed fair even if the consequence are, in the end, bad (and vice versa)
- Therefore animal experiment is bad even if it brings some good (drugs), because some evil (animal suffering) is generated

Protection of the laboratory animal: how?

By complying with regulations

- These laws are the expression of the will of civil society
- They deal mainly with care, treatment, housing conditions

By following ethics in animal experimentation

- Theory: the "3 Rs" and derived material
- Another level is the expression of a personal will (of the individual and/or of its institution)

In practice

- The site veterinarian
- The Institutional Animal Care and Use Committee / Animal Ethics Committee

Focus: origins of the protection of the laboratory animal

Reactions of the civil society against cruelty toward animals

- Passionate judgments: strong conviction, antagonism,"vivisection"; no discussion possible (Brigitte Bardot speaks of "genocide" animal in Canada in 2006, for the baby seals)
- Moderate judgments: usefulness is recognized but experimentation must be controlled and supervised (ultimate goal is to eliminate all animal experimentation)

The legislator answers to these reactions

- Definition of the legal conditions
- Contacts and communication with the moderate associations

The theoretical framework: the "3Rs"

Russell & Burch, 1959. The principles of humane experimental techniques

- Milestone book, with a rich theoretical apparatus developed since 50 years

Replacement

- Substitute higher animals by phylogenetically lower animals or by alternative methods (the original text says "an insensitive material")
- Development of models (BCOP to replace Draize test; Hepatocyte cultures; Ames test…)

Reduction

- Reduce the number of animals used
- Under the condition that the quantity and accuracy of information are maintained
- Especially, verify that the results are not already available and have good practices which give confidence in the results generated and which avoid the need to redo the study

Refinement

- Decrease the incidence or severity of inhumane procedures
- The protocol must be as complete as possible, including as much parameters as necessary and possible
- Fight against pain (know the physiology and the procedure)
- Define endpoints if the values set for the end points are exceeded

Additional Rs:

- Responsibility of the operator (role of the internal management, of the site veterinarian, of the scientist, of the technician, of the animal handler; personal ethics; initial and continuing training)
- And Remembering

Focus: parameter [final point, outcome measure] and endpoint [stop point], surrogate, biomarker

- Parameter: criterion evaluated, criterion of efficacy
- Endpoint: when the parameter is outside the norm, requiring a decision for the well-being of the animal and for the study outcome
- Surrogate marker, biomarker

(More information on the endpoints and a list of criteria will be supplied during the lecture)

The site veterinarian

He is the specialist of the animal. Each institution should have one (at least part time). European regulations dictates specific training ("level D")

He must supervise the animal husbandry team, who are the first contact with the animals and who will first detect any change in conditions and well-being of the animals

The Institutional Animal Care and Use Committee (IACUC) / Animal Ethics Committee (AEC)

The role of the IACUC in several countries will be compared

(Personal comment: there is no Animal Ethics, only Ethics of Animal Experimentation)

Roles of a IACUC

Applying the regulations

- Improve the well-being of animals
- Supervize the supply, maintenance, handling as related to the animals
- Promote environmental enrichment
- Limit animal suffering by defining endpoints
- Monitor staff (checking initial and continuous training)
- Monitor study conditions, including sub-contractors

Applying the "3 Rs"

- Checking the studies are really necessary
- Review study protocol (advice is binding or advisory only, depending on the countries)
- Review procedures and techniques
- Advise on new methods to implement in order to fulfill the 3 Rs

Giving its opinion on any matter relating to the use of laboratory animal

- This advice may be advisory or binding, depending on the regulations

Setting the policy of the institution

Serving as information center if a crisis arises

Means of a IACUC

Constitution

- 1 president employed in the establishment

- 1 veterinarian (or specialist of laboratory animal)
- Staff, researchers and non-personality researchers
- 1 external member, representative of the social bodies (optional)

Meetings

- Initial review of standard protocols, procedures, techniques
- Consideration of their amendments review of all specific protocols

Free access

- The IACUC has free access to all premises related with animal experimentation
- The staff has free speech and free access, with anonymity, to the IACUC

Independence from hierarchy

- Reporting to the institution management

Added values of a IACUC

Improve the study quality

Sensitize to animal protection

Advice is required to publish in more and more scientific journals

Control the diffusion of the information, whether internally or externally

Conclusion

Starting point = the civil society expresses a request in favor of the laboratory animal

- Ethical thinking and legislative answers are put in place

By their additional thinking and their actions, scientists show they are responsible and respectful of the laboratory animal

The site veterinarian and the IACUC are the scientific and the regulatory watchmen, respectively, of the well-being of the laboratory animal

INVITED LECTURES ABSTRACTS

L02

Spitz Tumours

R. L. Barnhill, M.D.

Hôpital Saint-Louis, Paris, France

Over more than sixty years there has been an evolution in terminology that has often reflected the current thinking about the biological nature of this group of lesions. Thus various terms have included juvenile melanoma, epithelioid cell nevus, spindle cell nevus, spindle and epithelioid cell nevus, large spindle and/or epithelioid cell nevus, Spitz tumor, Spitz's nevus, and Spitz nevus. Although the biological nature of these lesions, particularly unusual variants, and those lesions difficult or impossible to differentiate from melanoma await more definitive characterization, there is a consensus that *Spitz naevus or Spitz tumour* is an acceptable term for this general category of lesions for the time being. While these lesions undoubtedly share biological properties with "conventional" melanocytic nevi, they are also at the same time distinctive melanocytic neoplasms, suggesting the need for some qualification such as spitzoid melanocytic tumor, neoplasm, or melanocytoma, particularly for those with atypical features. Distinctive variants that are generally accepted include desmoplastic Spitz nevi, pigmented spindle cell variants, halo Spitz nevi, combined variants, recurrent/persistent Spitz nevi, and finally Spitz nevi/tumors (or neoplasms) with deviant or atypical features. There is a general acceptance that often predominately or entirely dermal desmoplastic Spitz nevi are characterized by a desmoplasia (sclerosis or collagenization) of the dermis and sometimes subcutaneous fat that contains the spitzoid melanocytes. Such lesions show varying degrees of cellularity often with individual or small aggregates of melanocytes sequestrated in dense collagen. A proportion of these desmoplastic lesions commonly harbor a concurrent population of small ovoid melanocytes that may represent small conventional nevus cells or small spitzoid melanocytes resulting from maturation. The two populations of melanocytes may be closely intermingled or topographically distinct. There is a general agreement that pigmented spindle cell variants of Spitz nevus are distinctive and the term *pigmented spindle cell nevus* is acceptable to designate these lesions (see discussion below). Halo variants of Spitz nevi are defined by a distinct clinical halo and associated dense lymphocytic infiltrates. As discussed above Spitz nevi with two or more distinct populations of melanocytes are accepted as legitimate entities but probably require rigorous attention as to how they are defined. Recurrent or persistent Spitz nevi/tumors following apparent incomplete removal are now clearly recognized. However, considerable caution should be exercised in these circumstances as to the limitations of histological interpretation of such "recurrent" lesions without comprehensive evaluation of the individual lesion and patient and long-term follow-up of the patient for an adverse outcome. Finally there is general agreement that Spitz tumors (or neoplasms) with a spectrum of atypical or deviant features exist. However, the challenge to pathologists and clinicians alike is to accumulate sufficient knowledge about this spectrum of lesions so as to better or more fully understand their biological natures, develop appropriate terminologies, and management strategies for patient care. Unusual or atypical variants of Spitz tumor may demonstrate a number of morphological characteristics often observed in or mimicking melanoma or other atypical melanocytic lesions. What to make of such abnormal or deviant

morphological characteristics has remained the subject of considerable debate since their biological significance has remained largely

uncertain for many such ambiguous spitzoid lesions. One perspective holds that such abnormal findings are for the most part simply variations in the spectrum of Spitz tumors. On the other hand, another perspective maintains that the progressive accumulation of certain abnormal features may have some correlation, albeit imperfect, with neoplastic recurrence or progression of Spitz tumors. Thus methods have been suggested for the risk stratification of Spitz tumors exhibiting a range of abnormal properties. Controversial spitzoid lesions remain so poorly understood as to preclude any definitive statements as to their biological nature other than that they require rigorous study and in general neoplastic progression and an adverse outcome cannot be ruled out. Unusual, atypical, or deviant Spitz tumors may be characterized with respect to alterations of either the intra-epidermal components, dermal components, or both. Thus variants with striking pagetoid melanocytosis or pagetoid Spitz nevus/tumor and junctional configurations suggesting conventional atypical or "dysplastic" nevi may be observed on occasion. However, abnormalities of the dermal component have received more attention because of their potentially greater biological import. Consequently unusual Spitz tumors with significant dermal components manifesting asymmetry, large diameter (> 6 and especially > 10 mm), significant thickness (particularly subcutaneous extension), lack of "maturation" and nodule formation, and mitotic rates > 6/ mm2 (or in another study >2/mm^2) have been categorized as atypical or biologically ambiguous Spitzoid neoplasms. Other characteristics including age of the patient, ulceration, cytological atypia, and adjunctive studies such assessment of mitotic index, other biological markers, and analysis of chromosomal aberrations may aid in this evaluation.

INVITED LECTURES ABSTRACTS

L03

New classification of hepatocellular adenomas

P. Bioulac-Sage(1,2), C. Balabaud(2,3)

Services [1] d'Anatomie Pathologique, Hôpital Pellegrin, et [2] d'hépatologie, Hôpital St André CHU Bordeaux ; [3] Inserm, U889, Université Bordeaux 2, Bordeaux - France

Hepatocellular adenoma (HCA) is a rare benign tumor, occurring mainly in women taking oral conceptives. These tumors are rare in children, men and the elderly. Their incidence is around 3-4/100 000 in Europe and North America (1). In addition to oral conceptives, other risk factors have been identified including androgens, glycogenosis, tyrosinemia, familial polyposis coli.

HCA can be discovered by chance, but two main complications may occur such as bleeding (20-25% of cases) and rarely malignant transformation in hepatocellular carcinoma (HCC). Haemorrhage are mostly observed in HCA larger than 4-5 cm; they can occur inside the tumor, usually admixed with necrotic changes, or can lead to hemorrhagic rupture which can cause sub-capsular haematoma and possible haemoperitoneum.

HCA are composed of hepatocytes which are arranged in liver cell plates that are only mildly thickened or irregular. The tumour parenchyma is supplied by numerous arteries which are unaccompanied by bile ducts. Tumour hepatocytes are of normal size; the cytoplasm may be either normal, clear (glycogen-rich) or fatty. Nuclear atypia, mitoses are distinctly unusual. In contrast, changes including sinusoidal dilatation, peliosis, infarcts, and haemorrhage are frequent, and may result in oedematous or fibrotic regions.

The diagnosis of HCA can usually be made on routine staining but pathological variations are frequently seen and immunohistochemistry is becoming essential for classification into different subtypes (2), whose recognition is important for the follow-up and management of the patient (3).

The HCA subgroups according to genotype / phenotype classification

HCA are monoclonal tumours in which several recurrent mutations have been identified (4-7).

They represent a heterogeneous entity, recently sub-classified according to their genotype and phenotype. This classification was proposed following a large multicentric French study of molecular markers of HCA and their correlation with microscopic pathologic features (4). In our institution more than 130 HCA resected between 1984 and 2008 have been reviewed and classified according to genotype/phenotype and immunohistochemical characteristics (3).

1- HCA with mutations in the HNF1A gene: H-HCA

HNF1A (also called *TCF1)* gene encodes the hepatocyte nuclear factor 1 (HNF1) . Biallelic inactivating *HNF1A* mutations have been identified in approximately 40% of HCA; in most cases, both mutations are of somatic origin, whereas in less than 10 % of cases, one mutation

is of germline origin while the other is somatic. Somatic H-HCA occur almost exclusively in women. Patients with germline *HNF1A* mutations are younger than those with somatic mutations and

they frequently have a family history of liver adenomatosis (8); they may or may not have clinical diabetes, which is usually of the MODY3 type. Recently, CYP1B1 heterozygous germline inactivating mutations were identified in 15% of women that have *HNF1A*-mutated HCA, suggesting that germline CYP1B1 mutations may also confer predisposition to the development of H-HCA (9).

H-HCA mostly correspond to a histologically homogeneous group of tumours, characterised by marked steatosis, no cytological abnormalities, no inflammatory infiltrates. Furthermore, *FABP1*, which is a gene positively regulated by *HNF1A*, and expressed in normal liver tissue, displays downregulated expression levels in H-HCA. Correlatively, using immunohistochemistry we observed a lack of LFABP expression in the tumour which contrasted with normal expression in the surrounding non-tumoral liver (2). The sharp contrast between tumor and adjacent liver in terms of steatosis and LFABP expression enables delineation of tumor borders which are often irregular and lobulated, with often small HCA foci present in the immediate surrounding parenchyma. When liver adenomatosis is observed, multiple adenomas of varying size are present, associated with a myriad of steatotic micronodules. All these micronodules can be correctly identified by lack of LFABP staining.

2 - HCA with mutations of the β-catenin gene: β-HCA

β-catenin mutation leads to the activation of the Wnt/ β-catenin pathway that plays a key role in liver development and physiology. Approximately 10-15% of HCA (4, 6) demonstrate an activating β-catenin gene mutation.

β-HCA are usually characterised by the presence of cytological abnormalities and acinar pattern, whereas they are not steatotic (4). Due to the cytological and architectural abnormalities, these tumors are often extremely difficult to distinguish from well differentiated HCC. Moreover, they are also more frequently associated with the development of unequivocal HCC than other HCA subtypes (2-4, 10). Furthermore, this subgroup of β-HCA is over-represented in male patients and specific risk factors are often found, such as male hormone administration, glycogenosis, and familial polyposis.

Activation of the Wnt/β-catenin pathway can be demonstrated by immunohistochemistry; the usual pattern results in an aberrant nuclear and cytoplasmic staining, distributed in a random and heterogenous pattern. Furthermore, Glul, a β-catenin target gene, coding for glutamine synthetase, also displays upregulated expression, with usually a strong and diffuse staining pattern, often easier to interprete than β-catenin staining (2).

3- Inflammatory hepatocellular adenomas: IHCA

IHCA account for more than 50 % of all HCA. They are characterised morphologically by the presence of inflammatory infiltrates, varying degrees of sinusoidal dilatation or congestion, numerous thick-walled arteries, and a more or less obvious ductular reaction, lying inside a small amount of connective tissue (4). This subgroup of HCA include most of the previously termed "telangiectatic focal nodular hyperplasia" (11) or telangiectatic adenoma (12). In IHCA, there is an overexpression of molecules of the acute phase inflammatory response, such as serum amyloid A (SAA) and C reactive protein (CRP) at both the mRNA and protein

levels (2). Therefore, immunohistochemical detection of these proteins (SAA and CRP) in tumoral hepatocytes with a sharp demarcation from the surrounding non tumoral liver is a very good argument for the diagnosis of IHCA (2). Steatosis may be present in IHCA, but is usually not as extensive as in the H-HCA.

It has recently been shown (7) that approximatly 60% of IHCA are associated with gain-of-function mutations caused by in-frame somatic deletions in the *IL6ST* gene. This gene encodes for the signalling co-receptor gp130. Mutant gp130 constitutively activates STAT3 signalling in the absence of IL6 binding.

IHCA are significantly associated with high body mass index and alcohol consumption (3, 10, 11). These patients may have signs and symptoms of an inflammatory syndrome, including elevated serum CRP levels which may regress after IHCA resction (13).

Approximately 10 % of IHCA have also a mutation in the β-catenin gene. The risk of HCC does also exist in these inflammatory/ β-HCA, but in a proportion that is presently unknown.

4- Hepatocellular adenoma without markers

Tumors in this group, representing less than 10% of cases neither show *HNF1A* nor β-catenin gene mutations and do not express inflammatory proteins.

Differential diagnosis

Differential diagnosis may be challenging, mainly in a liver biopsy; in particular the distinction of FNH versus HCA, HCA versus well differentiated HCC, steatotic FNH or steatotic HCA versus focal fatty changes and even FNH or HCA versus normal liver. Immunohistochemistry as detailed above is very useful in these situations provided that tumoral and non tumoral liver tissue is available for comparison. Although glypican-3 is a promising marker for the identification of HCC, its utility is limited by the fact that it may not be detected in approximately one third of HCC which develop in a normal liver.

Furthermore, the two lesions, FNH and HCA may co-exist (14), especially when there are multiple nodules eg, adenomatosis and FNH, or multiple FNH and HCA. Errors are best avoided by assaying a panel of immunohistochemical markers (2, 15) and interpreting them in the appropriate clinical, biological and radiological context with the benefit of expertise from a team with a special interest in these hepatocellular nodules. It is always careful to freeze tissue for molecular charaterization, particularly when β-catenin mutation is suspected.

Finally, the number of detectable HCA is often less that the true number of HCA since microadenomas are not detected by conventional imaging. The term adenomatosis – if kept – which should mean many HCA, is not a specific type of HCA as thought previously (16-18). From a clinical perspective, size, genotype, and underlying disease is far more important than number.

The respective role of imaging and needle biopsy for the diagnosis of HCA subtypes is improving. We have shown that H-HCA and IHCA were associated with specific MRI patterns related to diffuse fat repartition and sinusoidal dilatation, respectively (19). When imaging techniques fail to identify liver nodules, then comes the place for liver biopsy where immunohistochemistry could be also a promising tool for helping to identify the different types of nodules. The ultimate result will be given using the surgical specimen.

Conclusion

Taking into account noticeable differences between the HCA subgroups, in terms of clinical and prognostic features, phenotyping may become an important tool for HCA management strategy. However, the importance of the genotype/phenotype classification needs to be confirmed by additional studies particularly from teams of other countries where the use of oral contraceptives is less common.

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INVITED LECTURES ABSTRACTS

L04

Papillomaviruses in equine skin lesions

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Papillomaviruses are recognized as causal agents of a variety of proliferative diseases in many species. In horses, equine sarcoid is the most common tumor and is caused by bovine papillomavirus type 1 and 2. Recently, genital and ocular squamous cell carcinomas have been linked to equine papillomavirus type 2 infection. Other papillomavirus induced diseases in horses, such as cutaneous papillomas and hoof canker will be briefly discussed.

Equine sarcoid

Equine sarcoids are the most prevalent tumor in horses, with up to 12% of the population being affected. Clinically and pathologically, equine sarcoids present most of the features of a true neoplasm. The predominant cell type is a malignant/transformed fibroblast. Sarcoids generally have a high capacity for local tissue invasion into the dermis and subcutis but true metastatic dissemination does not occur. The clinical presentation of an equine sarcoid can vary a lot between different individuals and within the same individual. The number of tumors per horse varies from one single sarcoid over a few to more than 100 lesions. A sarcoid can remain stable for years or it can show a rapid and aggressive growth with infiltration of surrounding skin. Sarcoids themselves are never lethal, but failure to treat them and the functional impairment they cause may result in euthanasia.

At cross-section sarcoids appear as a dermal thickening with a pale yellow color and a firm texture due to fibroblastic proliferation and a small number of capillaries within the tumor. Several types have been described, both clinically and histologically. Verrucous sarcoids have a typical wart-like appearance with a rough, thickened, scabby surface above the fibroblastic part of the tumor. They can appear as small exophytic growing masses or as flat, often extended, scaly areas of skin with multiple smaller wart-like lesions on the surface. Nodular sarcoids are subcutaneous, easily moveable nodules, often but not always spherical, covered by intact, apparently normal skin. In some cases, however, the overlying skin can be thinner, shiny and adherent to the tumor. Occult sarcoids are flat circular to oval areas characterized by alopecia and a roughened or scaly appearance. Sometimes small cutaneous nodules can be observed. In some cases, occult sarcoids can be very subtle, showing no more than a slightly thickened skin with a thin hair coat and slight changes in pigmentation. This relatively benign type can evolve rapidly towards a more aggressive type, either spontaneously or following injury such as biopsy or inadequate treatment. Fibroblastic sarcoids are large fibrous masses with an ulcerated surface. This aggressive sarcoid type can

evolve from any other type after accidental or iatrogenic manipulation, including biopsy. Malevolent sarcoids are a very rare type that infiltrates in lymphatic vessels resulting in multiple nodular or fibroblastic masses along these vessels. Local lymph nodes might also be involved. Sarcoids infiltrating the underlying muscles have occasionally been observed. Mixed sarcoids are a combination of two or more of the above mentioned types. Equine dermal neoplasms histologically mimicking Schwannomas, but lacking the interaction with the epidermis which is typical for equine sarcoids, have been confirmed by BPV-PCR to be a previously unrecognized presentation of equine sarcoid.

Typical histopathological changes are subepidermal proliferation of spindle-shaped to fusiform fibroblasts showing hyperchromasia with a moderate to high cell density. These immature fibroblasts show a higher density in the superficial part of the tumor compared to the deeper layers. The fibroblasts are fusiform to spindle-shaped, forming whorls, interlacing bundles and haphazard arrays with one another. At the dermo-epidermal junction fibroblasts are oriented perpendicular to the basement membrane, which is known as a picket-fence pattern. The mitotic rate is invariably low. The amount of collagen varies considerably between tumors. In between the fibroblasts of the deeper layers of the dermis, large polygonal cells with large basophilic nuclei can be found. These are infiltrating dendritic cells that have an antigen presenting function suggesting an immune mediated defence against virus infected cells. Sometimes small epithelial inclusion cysts are found. In the most typical cases, pseudoepitheliomatous hyperplasia and hyperkeratosis are observed. There is marked formation of rete pegs, which are broad invaginations (up to more than 20 cells) of epidermal cells into the dermis. However, in less typical sarcoids the epidermal component can be normal, atrophic or even absent. The histological properties seem to be mainly dependent of the clinical type. Most of the verrucous and mixed sarcoids display the histological features as described above. In verrucous sarcoids, the epithelial component is much more important than the dermal, sometimes only lying as a small band of active fibroblasts against the epidermis. In nodular sarcoids the epidermis is often thinned. If rete pegs are present, they are short. If the dermal proliferation is not making contact with the epidermis, the latter is normal. In occult sarcoids the epidermis is often normal or only displaying slight changes. An increased density of subepidermal fibroblasts infiltrating between a reduced number of hair follicles and sweat glands can be observed. They do not show a typical morphology or specific whorling distribution pattern. The density of dermal fibroblasts is lower compared to the other types of sarcoids. Some occult sarcoids show focal dysplastic epithelial changes, comprising focal acanthosis composed of swollen keratinocytes with slightly basophilic, pale staining cytoplasm, and perinuclear halo (koilocyte-like change) often associated with a thickened hyaline basement membrane and without dermal changes. In the fibroblastic type there is always partial or total ulceration of the epidermis with infiltration of polymorphonuclear cells. The epidermis just next to the ulcerative lesions is more or less hyperplastic.

Bovine papillomavirus (BPV) type 1 and 2 plays a major role in the pathogenesis of equine sarcoids. Papillomaviruses are normally strictly species-specific, but equine sarcoids result from a natural cross-species infection. Whereas a typical papillomavirus infection initiates in the basal layers of the epithelium, with formation on new infectious particles alongside with epithelial maturation, BPV infection in horses predominantly occurs in the subepidermal fibroblasts, with no or minimal virion production. Analysis of early stage sarcoids, such

as occult sarcoids and latently infected normal skin, shows BPV infection in the epithelial layers. This indicates that BPV infection might start in keratinocytes, followed by a transition to fibroblasts allowing the virus to fully exert its transformational capacities.

Equine sarcoids can be diagnosed in three ways: clinical examination, histopathology, and detection of BPV DNA. A thorough clinical examination combined with a focused anamnesis (duration of problems, localization of lesions, age, breed, evolution, multiplicity of lesions…) can be sufficient in many cases. Lack of clinical experience or atypical tumor characteristics may cause confusion and necessitate lab-assisted diagnosis. Histopathological examination is often diagnostic, but it should be remarked that taking a biopsy, in particular of small stable lesions, may induce rapid growth and ulceration. If a non-excisional biopsy must be performed, sites within the mass must be carefully chosen to minimise the confounding factors of surrounding inflammation and granulation and to include intact epidermis. Possible deterioration of the sarcoid following biopsy is the reason why taking a biopsy is contra-indicated, even if making a definitive diagnosis is not possible in such cases. Another possibility is to perform a full surgical excision as if the lesion was a sarcoid, including excision of wide margins of normal skin and non-touch approach, followed by histological confirmation afterwards. This allows the pathologist to observe the range of morphological characteristics of the tumor allowing a correct diagnosis. Another approach in diagnosing equine sarcoids is the detection of BPV DNA in lesions using

polymerase chain reaction (PCR). This can be performed on paraffin embedded tissue suspected of equine sarcoid but not displaying the typical histological features. PCR for BPV is strongly recommended in all equine skin tumors that have Schwannoma characteristics. BPV PCR can also be performed on the limited amount of material obtained by swabbing or scraping the lesions. This technique is especially valuable for the diagnosis of equine sarcoid tissue in non-healing wounds and in case of recurrences after former surgery. PCR detection of BPV DNA has many advantages: it is not invasive, sampling is easy and the trauma to the tumor is minimal. Disadvantages are the lower sensitivity compared to clinical and/or pathological diagnosis and the presence of latent BPV in equine skin without causing pathology. Studies in our lab have found a widespread occurrence of BPV in the horse population, with 50 to 75% of horses, living in close contact with affected animals, being infected. However, the viral load is substantially lower in these cases. In conclusion, a correct and reliable diagnosis of equine sarcoids may require a combination of different approaches.

Squamous cell carcinoma

Squamous cell carcinoma (SCC) represents 20% of all equine tumors, making it the second most common neoplasm in horses. SCC is a malignant epithelial tumor and is most often associated with the eyes and the external male genitals, but it can develop in any epithelial tissue of the body. In many horses, penile SCCs are accompanied by large, confluent, pink to yellowish plaques, often referred to as precancerous lesions or penile intraepithelial neoplasia (PIN). Additionally, many genital SCCs are accompanied by genital papillomas.

Histological examination of PIN lesions shows plaque-like thickening of the epidermis due to irregular epithelial hyperplasia and formation of deep and broad rete ridges. The affected epidermis shows moderate to severe dysplasia throughout all layers of the epidermis with lack of orderly maturation. Keratinocytes are highly pleomorphic and their appearance

varies from small and polygonal with oval or flattened hyperchromatic nuclei to large and round with a pale cytoplasm and large vesicular sometimes irregular nuclei. Occasional mitotic figures are present within all epidermal layers. Multifocal groups of koilocytes can be observed. There is no evidence of local invasion and no basement membrane disruption. Well differentiated SCCs have numerous keratin pearls and obvious intercellular bridges. Moderately differentiated SCCs show individual cell keratinization, occasional keratin pearls and poorly defined intercellular bridges. More aggressive tumors show a highly invasive growth affecting the underlying tissue. These tumors usually show numerous individual neoplastic cells or small tumor nests infiltrating the surrounding connective tissue and can be associated with metastases. SCCs induce a moderate to severe inflammatory reaction characterized by abundant infiltration of lymphocytes, plasma cells and macrophages.

Recent research in our and other labs has demonstrated a novel equine papillomavirus (EcPV2) in all genital SCCs, PIN and papillomas, and in a subset of ocular SCCs. EcPV2 DNA has also been demonstrated in a single case of nasal SCC, as well as in draining lymph nodes of a penile SCC. Additionally, latent EcPV2 infection in normal genital (including cervical) and ocular equine mucosa was demonstrated in healthy patients.

Cutaneous papilloma

Equine papillomas are caused by equine papillomavirus type 1 and can be easily diagnosed clinically. Because of the absence of alarming side effects, they are therefore seldom submitted for histological examination. Cutaneous papillomas represent only about 5% of all equine neoplasms submitted to diagnostic laboratories.

Equine papillomas appear as small, elevated, circumscribed, heavily pigmented, horny masses, 2–20 mm in diameter, and range in number from 2 to more than 100. Histologically, these lesions consist of marked epithelial proliferation on a thin fibrovascular stalk in a papillary pattern. Intranuclear inclusions have been found within cells in the stratum granulosum that have abundant clear cytoplasm and numerous large, dark, basophilic, keratohyalin-like cytoplasmic granules. Cutaneous papillomas are found in horses 1–2 years old, especially if they have been in contact with other young horses. The infection often spreads from contaminated halters to the muzzle and then to the legs because horses frequently rub their legs with their noses to dislodge flies. Papillomas usually persist for 1–9 months and then regress spontaneously. Horses develop complete immunity following infection.

Aural plaque

Aural plaques occur in horses of all ages, manifesting as well-demarcated raised, depigmented, hyperkeratotic plaques on the inner surface of the ear pinnae. They rarely, if ever, spontaneously resolve and are susceptible to fly bites and secondary infection. The main histopathological findings are epidermal hyperplasia and hypomelanosis with abrupt change between the normal and the affected epithelium. Immunohistochemical analysis suggests the involvement of papillomavirus infection, but the causal agent has not been identified yet.

Hoof canker

Recently, a possible link between BPV and hoof canker has been suggested. In one study, BPV was detected in 11 horses with hoof canker, not only in the hoof canker lesions but also in normal skin biopsies and peripheral blood samples of a subset of these horses. A small

study in our department confirmed BPV presence in hoof canker lesions of 5 horses. A causal mechanism however still remains to be determined, and it is clear that other factors, such as bacterial infection and bad hygiene also play a role.

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INVITED LECTURES ABSTRACTS

L05

Life Long Learning, the European Pathology continuous assessment programme

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Although the practice of pathology in Europe and around the world is quite similar, the methods and contents of specialty training and the expected competencies and their evaluation at the end of the training program (and therefore also during active professional life), vary significantly. With the free movement of doctors within the EU these differences have been a source of concern since its foundation in 1957. Countries with very demanding training programs are often reluctant to hire doctors from countries with less well defined training requirements. Harmonisation of the training programs has therefore be the goal of many efforts in the past.

The last two decades have shown that the harmonisation of training is difficult to achieve by attempting to reach a consensus about a more homogeneous approach regarding the training programs (1, 2). National certifying organisations and practices preclude such a solution at the present time. However, consensus can be reached more easily on training outcomes and the competencies required of a medical specialist. These competencies can be evaluated and their inclusion in training programmes assured through assessment programs. The goals of the Life Long Learning programme are to define which competencies are expected of pathology trainees in Europe at the end of their specialty training and to provide pathologists with tools to subsequently remain competent for the rest of their career. No matter where one is trained and under which conditions, common end terms can be defined for all European countries and many of these competencies can be tested. A universally accepted competency test, that might ultimately function as a European Boards Examination in Pathology, can assure that training outcomes are similar and will facilitate the movement of (junior) doctors around the European continent. It could also contribute to specialty-specific evaluations in those countries that develop formal evaluation programs.

To achieve this goal a European network was formed with 39 official partners, all academic pathology chairs and/or program directors. This was achieved within the framework of the European Association of Pathology chairs and Programme directors (EAPCP, 3). In addition, more than 200 others with the same background have expressed interest in the program. This consortium represents nearly all European academic pathology institutes with training facilities and active training programmes.

The main achievement of this first period of the Life Long Learning program has been the development of a document describing the profile of the discipline in Europe and the general characteristics of a training programme, in terms of content sufficiently detailed but in structure open enough to allow for individual countries and training institutions to adapt the programme to the local situation. Furthermore, agreement was reached on the general

and pathology-specific competencies at the end of training. This has been realised in the document "*A European Pathology Curriculum*", especially written for this occasion. The above mentioned academic departmental chairs and program directors were consulted and they approved the contents.

In addition, these competences were used to prepare a web based test using virtual microscopic slides: A trial run was conducted in October 2008, of which the primary aim was to assess IT feasibility of the approach: access of between hundreds and even thousands of participants simultaneously to a (network of) server(s) on which the test was offered. Essential experience was gained and important steps were set into the direction of a robust stable IT infrastructure. A second test was subsequently conducted in a small group of participants to fine tune details of the IT infrastructure. The first real time test was run on October 2009. The general appreciation of the test was very positive, regarding both the general approach and the content and the level of complexity of the test items. The presently available data show that a fully functional system is within reach. The IT structure still might need improvements in order to provide an optimal test environment for virtual microscopy test items. It is clear that an easily accessible stimulating test environment without technical hiccups is within reach. Close coordination with national bodies for the accreditation of medical specialists and the UEMS will be necessary to fit this approach into a coordinated and generally accepted competency validation approach.

Information about the program is available on the website: http://www.eapcp.org/Europals/europals.htm.

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INVITED LECTURES ABSTRACTS

L06

General principles and interpretive guidelines for Her2 FISH

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Approximately 10 to 25% of breast cancers overexpress Her2 protein, usually as a result of amplification of the Her2 oncogene on the long arm of chromosome 17. Assessment of Her2 status has become crucial for selecting patients for Her2 targeted therapy (trastuzumab; Herceptin), both in the adjuvant and the metastatic setting. Currently, most laboratories in Belgium use immunohistochemistry (IHC) as a primary test for detection of Her2 overexpression on the cell membrane. In the event of an IHC score 2+ or 3+, fluorescence in situ hybridization (FISH) has to be performed, since treatment with trastuzumab is only reimbursed when amplification of the Her2 gene is proven by FISH. Therefore, FISH testing for determination of Her2 status has become essential in diagnosis and treatment of breast cancer patients.

This summary will focus on general principles of fluorescence in situ hybridization and on interpretive guidelines for Her2 FISH, including controversial issues as 'polysomy 17' and 'genetic heterogeneity'.

GENERAL PRINCIPLES

In situ hybridization (ISH) is a molecular technique which utilizes DNA or RNA probes to assess intact cells for various types of genetic alterations as translocations, amplifications and deletions. If ISH is performed with fluorescent-labeled probes, the technique is referred to as Fluorescence In Situ Hybridization, or FISH. This technique has found many important applications in oncology (breast cancer, hematological malignancies, soft tissue sarcomas).

To understand how FISH works, one should be familiar with DNA structure, principles of base pairing, denaturation and hybridization. DNA consists of two long chains of nucleotides held tightly together in a complex structure known as the DNA double helix. These two chains are bound together by base pairs. The nucleotides that comprise DNA contain one of four bases: adenine (A), cytosine (C), guanine (G) or thymine (T). In double-stranded DNA, it is the pairing of A's to T's and C's to G's that keeps the two strands of DNA bound to one another through hydrogen bonds. Denaturation refers to the process of making double-stranded DNA into single-stranded. Denaturation is brought about by breaking the hydrogen bonds that hold the two strands of DNA together. This is most commonly achieved by applying heat (i.e., raising the temperature of the sample, 'melting' the DNA). In addition, certain chemicals such as formamide can be used to promote denaturation. Hybridization occurs when two strands of complementary single-stranded DNA molecules stick to one another. The more these two single-stranded molecules complement each other, the more likely those strands will hybridize. In situ hybridization utilizes double-stranded DNA (or single-stranded RNA) probes that are designed to hybridize to specific sequences of base pairs on cellular/target DNA. DNA probes that are commonly used for oncologic applications are chromosome enumeration probes (CEP) and locus-specific indicator (LSI) probes. CEP probes hybridize to repetitive DNA sequences found near the centromeres of chromosomes. These probes, specific for the centromere of a given chromosome, are used to enumerate the number of copies of a given chromosome in a cell. LSI probes hybridize to unique nonrepetitive DNA

sequences and are generally used to determine if specific genes are amplified, deleted or translocated.

Probes that are used for FISH are labeled with a fluorophore. A fluorophore is a molecule that fluoresces when excited by light of specific wavelength. The commonly used CEP and LSI probes are generally labeled with green and red fluorophores, respectively.

The steps involved in performing FISH are as follows. First, tissue sections of 4 to 6 µ are made from paraffin-embedded formalin fixed tissue. Secondly, these tissue slides have to be pretreated with a protease to increase the accessibility of double-stranded cellular DNA to probe DNA. The third step involves denaturing the probe DNA and cellular DNA at high temperature. Because of the heat, the double-stranded DNA becomes single-stranded DNA. In the fourth step, the temperature is lowered to about 37°C to allow the single-stranded probe DNA to hybridize to its specific single-stranded target DNA. This hybridization process takes several hours to complete and is usually done overnight. Following hybridization, the slide is washed with a specific washing solution. The goal of this step is to remove any probe that is not specifically bound to the desired target without removing the other probes. After the post hybridization wash, a solution which contains DAPI is placed on the slide. DAPI is a blue nuclear counter stain that allows one to see the nuclei. The hybridized slides have to be stored in a refrigerator and in the dark because fluorophores have tendency to fade with exposure to heat and light. The last step involves an assessment of the fluorescent probe signals in the nuclei with a fluorescence microscope.

INTERPRETIVE GUIDELINES

Probes used in Her2 FISH assays are the CEP17 probe and the Her2 DNA probe. The CEP17 probe hybridizes to the centromeric region of chromosome 17. The Her2 DNA probe is a LSI probe specific for the Her2 gene locus on the long arm of chromosome 17. The CEP17 probe is labeled with green fluorophores and the Her2 DNA probe is labeled with red fluorophores. This allows one to see green and red signals in the nuclei (hence the name 'dual-color FISH'). The green signals are assumed to be a marker of ploidy status (normal versus increased number of chromosome 17), whereas the red signals reflect the Her2 gene status of the cell (normal versus increased copy number of Her2 gene). A normal cell in rest harbors 2 copies of chromosome 17 and 2 copies of Her2 gene, resulting in 1 to 2 green signals and 1 to 2 red signals. One does not always see 2 signals of each color because of nuclear truncation or because the signal is in different planes of focus. In the event of Her2 gene amplification up to 30 gene copies can be found in the nucleus, which results in a cluster of red signals. The CEP17 probe is used to correct absolute Her2 gene copy number with the number of chromosome 17, in order not to misinterpret an increased number of chromosome 17 ('polysomy 17') as Her2 gene amplification.

According to the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) guidelines for Her2 testing, at least 20 non-overlapping nuclei of invasive tumor cells should be counted in 2 different areas of the tumor. It is recommended to scan the entire invasive tumor because of the possibility of tumor heterogeneity. For each nucleus, the number of Her2 gene signals and the number of CEP17 chromosome probes should be scored. Based on these numbers, the average number of Her2 gene signals and the average number of CEP17 signals can be calculated.

A positive FISH test for Her2 is defined as a ratio average number of Her2 signals/ average number CEP17 signals of more than 2.2, whereas a negative FISH test is defined as a ratio Her2/CEP17 of less than 1.8. When the ratio is between 1.8 and 2.2, the result is equivocal. A normal cell in rest will have a ratio of 2/2, which is a negative result. When a tumor cell harbors 15 copies of Her2 gene and a normal number of chromosome 17, the ratio is 15/2. This test is positive as a result of Her2 gene amplification. Unfortunately, interpretation of FISH assays will be much more problematic in tumors showing an increased copy number of both the Her2 gene and the chromosome 17 centromere. Indeed, a tumor cell harboring an increased number of chromosome 17 ('polysomy 17') will theoretically also contain more Her2 gene copies. The term 'polysomy' has been widely used and defined as ≥ 3 copies of chromosome 17 centromere (CEP17). This implies that the centromere is assumed to be representative for the entire chromosome 17. However, recent studies have shown that what one interprets as an increase in chromosome 17 (an increase in green signals) is mostly not a true polysomy, but a co-amplification of the centromeric region. According to the guidelines tumors showing an increased copy number of both the Her2 gene and the chromosome 17 centromere, but with a ratio less than 1.8 are considered as polysomic/ negative. In other words, it might well be that these tumors actually harbor true Her2 amplification, but are considered negative by FISH due to an increased number of CEP17 signals. Therefore it is important not to classify all the cases with an increased number of CEP17 signals as polysomic, but also take into account the mean number of Her2 signals and the pattern of Her2 signals. A high number of Her2 signals (> 6) clustered together should be classified as Her2-amplified, irrespective to the number of CEP17 signals. The importance of recognizing a true Her2 amplification despite the presence of increased CEP17 signals lies in the fact that tumors considered as Her2-amplified are treated with trastuzumab, whereas those considered as polysomic are not.

Recently an expert panel of the CAP has proposed a definition for intratumoral heterogeneity of Her2 gene amplification and practical guidelines for examining and reporting in these cases. They defined Her2 genetic heterogeneity (GH) as more than 5% but less than 50% of infiltrating tumor cells with a ratio higher than 2.2. Genetic heterogeneity can be detected by FISH by scanning the entire slide. According to the ASCO/CAP guidelines 2 areas of invasive tumor should be analyzed. If an area appears to have amplified Her2 signals, that area should be counted. Within these areas, cells should be selected randomly and a ratio of Her2/CEP17 signals should be calculated from all cells scored within this random population. The analyst should pay attention whether these amplified cells are present as scattered cells or in a specific cluster. If a cluster of at least 20 highly amplified cells is present, its ratio should also be specifically and separately provided, and a comment on whether the amplified area is histologically distinctive from the rest of the invasive tumor. At present, however, the clinical significance of GH in terms of the potential benefit from trastuzumab therapy remains unclear.

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INVITED LECTURES ABSTRACTS

L07

A new chicken model for human inflammatory bowel disease?

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1. Human inflammatory bowel disease:

Inflammatory bowel disease (IBD) is characterised by chronic inflammation of the lining of the gastrointestinal tract, which causes severe watery and bloody diarrhoea and abdominal pain. The onset of IBD is often at young age. Two different conditions fall under the collective term IBD, being Crohn's disease (CD) and ulcerative colitis (UC), and these entities are differentiated by a different pathogenesis and inflammatory profile (Papadakis and Targan, 2000). Whereas Crohn's disease can affect each part of the gastrointestinal tract, ulcerative colitis is usually confined to the colon and rectum. The incidence of inflammatory bowel disease (IBD) is clearly emerging and amounts to 20/100.000 in Europe and North America. Although the exact etiopathogenesis of IBD is not clear, it is widely accepted that the disease emerges from an inappropriate immune response in genetically susceptible individuals as the result of complex interactions among environmental (nutritional) factors, microbial factors and the intestinal immune system (Danese and Fiocchi, 2006). A 'hygiene hypothesis" suggests that a relative lack of contact with microbial antigens early in life would establish an immune system that is not primed to handle new challenges later in life and would generate an ineffective immune response that is prolonged because it cannot eliminate the offending agents (Bach, 2002; Danese and Fiocchi, 2006). Many other environmental factors have been proposed to be predisposing factors for IBD. Genetic factors also clearly play a role in the susceptibility for IBD. The strong influence of genetic determinants in CD has been shown by familial clustering (Peeters *et al.* 1996), and the high concordance rate in monozygotic twins (Halfvarson *et al.* 2003). First-degree relatives of affected individuals show a 20 to 50-fold higher risk for developing CD (Monsen *et al.* 1991). Multiple chromosomal regions have been linked with susceptibility to IBD (Barrett et al., 2008). The *CARD15* gene is the most understood susceptibility gene and explains about 20% of the genetic predisposition to CD (Hugot et al., 2001). CARD15 (or nucleotide-binding oligomerization domain 2, NOD2) is involved in the recognition of bacterial peptidoglycan-derived muramyl dipeptide (MDP) and triggers the production of pro-inflammatory cytokines and secretion of antimicrobial peptides.

It has become clear that an alimentary as well as a microbial component play an important role in the pathogenesis of IBD. Although certain specific micro-organisms, including *Mycobacterium paratuberculosis*, have initially been proposed to play a role in the onset of IBD, most studies have failed to find such an association. On the other hand, evidence has accumulated showing that the indigenous commensal microbiota is the target of the host immune response in IBD (McDonald et al., 2005). Compelling evidence comes from existing

animal models: genetically engineered rodents, such as IL10 deficient mice, develop colitis when exposed to commensal bacteria but remain disease free when raised in a sterile environment (Shi and Walker, 2004).

In addition, an increased number of bacteria is found in the mucosa of IBD patients (Swidsinski et al., 2002), and IBD lesions preferentially occur in segments with the highest bacterial concentrations (ileo-cecal valve and colon). Surgical diversion of the fecal stream prevents reappearance of CD whereas restoration of the fecal flow induces disease recurrence (D'Haens et al., 1998). Most IBD patients show enhanced immune responses towards bacterial antigens, one of which is flagellin (Lodes et al, 2004). It appears that the immune tolerance towards the commensal microbiota is diminished in IBD, as a consequence of defective bacterial sensing mechanisms, such as mutated CARD15 function (Girardin et al., 2003). Mucosal dendritic cell activation and increased levels of toll-like receptors (TLR) 2 and 4 are hallmarks of IBD, mediating an abnormal recognition of bacterial products (Hart et al., 2005). Also alterations in TLR3 and TLR4 expression by intestinal epithelial cells have been described in IBD (Cario et al., 2000). It seems that generalized overexpression of innate immune responses mediated by pattern recognition receptors (such as TLRs and NODs) is an important feature in IBD. CD is associated with a Th1 cell-mediated response, characterized by enhanced production of IFN-γ and TNF-α. UC is characterized by CD1 reactive natural killer T cell production of IL-13 and Th2 cytokine production (Sanchez-Munoz et al., 2008). In addition, many other non-immune cell types, including fibroblasts (as matrix metalloproteinase producers), endothelial cells and platelets are thought to play a role (Danese and Fiocchi, 2006). The end result is an excessive inflammation, leading to mucosal damage. It is remarkable that the focus of research in IBD has been on the immunological aspects of the disease, with only recently a rising interest in the microbiological aspects, and very little attention to the nutritional / dietary aspects.

2. A chicken model:

The purpose of the model was to study the potential adverse effects of diet on intestinal wall integrity in a genetically homogenous population of animals. For this purpose we used the following approach:

Commercial broiler chickens are genetically very homogenous, since they derive from crossing two or more inbred lines of chickens in a standardized way. It is well known among veterinarians that broiler chickens on a maize based diet thrive better than broilers on wheat, rye or barley based diets (Meng et al., 2004; Gracia et al., 2003; Lazaro et al., 2003). The latter cereals contain considerable amounts of non-starch polysaccharides (NSP), and it is generally accepted that these NSP have 'anti-nutritive' effects (Rubio et al., 1998; Choct and Annison, 1992), which can be overcome by the use of low dietary dosages of antimicrobials (commonly called growth promotors) (Choi and Ryu, 1987). The use of antimicrobial growth promotors in animal feed, however, has been banned in the EU since 01.01.2006.

NSP are known to increase the viscosity of the gut contents and have negative effects on nutrient digestion and absorption (Choct and Annison, 1992). The underlying mechanisms of the so-called "anti-nutritive" effects of cereals in general and NSP in particular have, however, not yet been investigated in detail. Nevertheless a good understanding of the pathological changes not only may help to control these problems in broilers but also may shed light on the role of the diet in persistent gut health problems in other animal species and in humans.

The purpose of the study was to analyse the alterations in the gut ecosystem (immune cell infiltration, gut morphology and microbiota composition) in relation to the cereal type (wheat/rye versus corn) used in the diet of broiler chickens.

The data from this study have recently been published (Teirlynck et al., 2009).

For this purpose, 800 newly hatched male broiler chickens, belonging to the genetically homogenous commercial line Ross 308, were used in the study. The animals were given 4 different diets (treatments), of which for each diet, 5 replicates (pens) of 40 chickens were used in the experiment. Forty chickens were housed per pen of 2.1 m^2 on a solid floor covered with wood shavings. Lighting in each room was controlled by time switch set to provide a 23 h light / 1 h dark photoperiod. Two treatment groups were fed a maize-soybean based diet of which one contained 100 mg/kg of the antibiotic growth promotor (AGP) Zn-bacitracin. The other two treatment groups were fed a wheat/rye-soybean based diet. Again one was supplemented with 100 mg/kg Zn-bacitracin. Each diet consisted of a starter, a grower and a finisher meal, all of which fulfilled all the known dietary requirements for broilers of that particular age group.

At 2, 4 and 6 weeks of age, all the broilers were weighed, feed intake was determined and feed conversion ratios (FCR) were calculated. At these time points also 5 chickens of each replicate were euthanized by intravenous embutramid injection. Samples of approximately 3 cm were taken from the second limb of the duodenum, the jejunum starting 1 cm after Meckel's diverticulum, the ileum immediately before the ileocecal junction, and the middle part of one caecum. These samples were fixed in formalin and embedded in paraffin. Also the content of jejunum, ileum and ceca was collected by rinsing according to Apajalahti et al.

Tissue sections were cut and stained with haematoxylin and eosin. Villus length in the small intestine was measured for five chickens of each replicate group, per treatment. This was done by random measurement of 15 villi per section (of all intestinal segments) using an Olympus BX61 Digital Camera DP50 (Olympus NV, Aartselaar, Belgium) and a pc-based image analysis system, Analysis® J-2 (P4 technologies, inc., Waldorf, Maryland, US). Adding Zn-bacitracin to the broiler diets increased the duodenal villus length significantly at 2 and 4 weeks of age, regardless of the cereal type.

Thickness of the tunica muscularis in duodenum, jejunum, ileum and ceca was also measured using the Analysis® J-2 software. For each section 9 measurements were performed on different locations. Measurements were done on cross sections of ring shaped intestinal segments which allowed unbiased perpendicular measurements. The tunica muscularis was significantly thicker in the group fed a maize-based diet compared with the animals fed a wheat/rye based diet (Table 5). Zn-bacitracin significantly increased the tunica muscularis thickness in all intestinal segments of the animals fed a wheat/rye based diet at week 2 and week 4.

The degree of villus fusion was determined using a scoring system (Teirlynck et al., 2009). The wheat/rye diet induced more fusion of villi compared with the maize diet. When both diets were supplemented with Zn-bacitracin, the effect of the cereal type was no longer statistically significant except in ileum at week 2, duodenum at week 4 and jejunum at week 6. Adding Zn-bacitracin to the wheat/rye diet generally decreased fusion of villi, while this was not the case in the maize-based diet.

Detection of goblet cells was done using Periodic Acid Schiff staining. More and larger goblet cells were present both on the villi and in the crypts at the age of 4 weeks in broilers

given a wheat/rye diet than in those given a maize diet. This was especially the case in the ileum and caecum.

Immunohistochemical labelling of leukocytes was performed as described by Mast et al. (1998) with monoclonal antibodies directed against T-lymphocytes (KUL05). The number of T-lymphocytes in the mucosa was scored with an automatic image analysis system (Optimas 6.5., Media Cybernetics, Silver Spring, USA), measuring the area percentage occupied by the labelled cells. These measurements were done in the propria mucosae. For each section, eight randomly selected sites were analysed by the image analysis program. From all 5 replicas of each dietary treatment, 5 chickens were analyzed in duodenum, jejunum, ileum and ceca. Immune cell aggregates were analysed using the sections stained for T-lymphocytes. In all 4 intestinal segments T-lymphocyte infiltration in the intestinal mucosa was generally higher in the group fed a wheat/rye based diet in comparison with the animals fed a maize-based diet (Table 6). Zn-bacitracin generally decreased T-cell infiltration.

Rehydrated, deparaffinized tissue sections (4 µm) were also used for detection of apoptosis in the crypts and at the tips of the villi. The In situ Cell Death Detection Kit, POD (Roche, Vilvoorde, Belgium) was used for Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL). More apoptotic cells were observed at the tips of the villi in the gut sections of the animals given a wheat/rye based diet, compared with animals given a maize-based diet. Zn-bacitracin reduced the number of apoptotic cells at the tips of the villi.

Samples of intestinal contents were analysed by t-RFLP. The jejunal, ileal and cecal content of the 5 chickens of one replica euthanised at the same timepoint were pooled. From these samples 1.0 g of intestinal or cecal material was taken. From these samples the DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen Inc., Hilden, Germany). PCR was run using primers amplifying 16S rDNA. The PCR products from the three replicates were pooled and purified with a QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany). The purified PCR products were digested overnight at 37°C with 20 U of *Hhal* (Boehringer, Mannheim, Germany) in 20 µl reaction mixtures. The fluorescently labelled terminal restriction fragment (T-RF) mixture was denatured at 95°C. Analysis of fragment size was performed on an automatic sequence analyzer (ABI 3130xl Genetic Analyser, 16 Cap., Applied Biosystems, Foster City, USA). T-RFLP fingerprint profiles of the microbial communities were collected by the software GeneMapper v. 3.7 (Applied Biosystems, Foster City, USA). DNA from different organisms will give rise to T-RF's of different size. A fingerprint of the community is visualized as peaks in an electropherogram. These fingerprints were transformed to band patterns by the use of BioNumerics (Applied Maths, St. Martens Latem, Belgium). The lengths of the fluorescently labelled fragments (each represented by one band) was determined by comparing them with the internal standard. After this the band patterns (presence or absence of bands) of the different samples were compared using BioNumerics. The comparisons were based on the Dice similarity coefficient and the un-weighted pair group method using arithmetic averages (UPGMA) for clustering. Dendrograms reflected the grouping and relatedness of samples. Clustering of the T-RFLP patterns of caecal samples of replicates of broilers given the same treatment was generally observed. All samples of the animals given a wheat/rye diet, either supplemented with Zn-bacitracin or not, clustered and showed a minimal similarity of 57%. The maize group replicates showed a minimum similarity of approximately 81%, the replicates of the Zn-bacitracin supplemented maize group show a minimum similarity of 73%. The cereal type had more impact on the composition of the microbiota, compared with supplementation of Zn-bacitracin to the feed.

In this animal model we showed that a change in diet induced a shift in the microbial composition in the gut associated with chronic inflammation and mucosal damage. The hypothesis of a microbial origin of the histological effects is supported by the fact that addition of bacitracin reversed the adverse effects of the wheat/rye based diet. It is questionable whether this chicken model is a good model for IBD in

humans. Nevertheless, the chicken model does point to a serious weakness in the currently existing animal models of human IBD, in the way that these models do not take into account the significant impact of ingredients used to formulate the feed. Indeed, different feed formulas fulfilling all nutritional requirements (energy, protein, amino acids, vitamins, minerals, etc) may still differ considerably in ingredient composition. Moreover, also in humans more research efforts should be put into the role of diet composition in IBD, either or not mediated through shifts in gut microbiota composition.

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INVITED LECTURES ABSTRACTS

L08

New insight in Papillomavirus-induced skin and mucous membranes carcinogenesis

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Papillomaviruses (PV) are small DNA viruses that induce a wide variety of skin and mucous membrane hyperplastic lesions. They are considered important carcinogens in humans and some high-risk PVs are directly responsible for the development of cervical cancers in women [1]. On the other hand, the role of PVs in the development of cutaneous squamous cell carcinoma (SCC) is not as definite [2]. There is however emerging epidemiological evidence to suggest that PV might play an important role in skin cancerogenesis, especially in epidermodysplasia (EV) associated-one. Evidence also suggests that ultraviolet (UV) radiation contributes to the cancerization of some PV-associated skin cancers [3]. Some animals models support the causative role of PV in the induction of skin SCC: A few decades ago, it was demonstrated that cottontail rabbit PV (CRPV) are able to induce skin cancers in rabbit [4]. Others studies have also established that attenuated life canine oral PV (COPV) vaccine induce SCC in Beagles [5]. Additionally, canine and feline can be affected by skin conditions that share some similarities with human EV and cancerization has been reported in some patients.

Epidermodysplasia verruciformis (EV) is a rare human genodermatosis characterized by multiple flat warts caused by so-called EV associated PV [6]. A major clinical complication of EV is the development of squamous cell carcinoma (SCC) which is reported in 30 to 50 % of affected people [7]. A similar condition is observed in immunocompromised people. Additionally, EV-like diseases have been described in the dog and cat [8, 9].

Canine pigmented viral plaques

A few years ago Nagata described cases of canine viral pigmented plaques and suggested that the condition could be the counterpart of human EV[8]. Fourteen cases of this condition have now been reported. Affected dogs usually present pigmented macules or slightly hyperkeratotic plaques. Carcinomatous transformation (In situ and invasive carcinomas) has been frequently reported in affected dogs (6 out of 14 cases).

Feline viral plaques

Feline viral plaques in older or immunosuppressed cats bear also similarities with human EV or canine pigmented viral plaques [9]. Dysplasia or atypia is not present but feline viral plaques often coexist in the same animal with bowenoid in situ carcinomas.

As PV antigens have been uncovered in both lesions on same animals, feline viral plaques could be regarded as precursory lesions of bowenoid in situ carcinomas (BISC) [10, 11]. Positive immunohistochemistry in BISC lesions supports a causative role of PV in the development of the lesions [12].

Skin SCC in dogs

A few years ago, Bregman and coworkers reported that attenuated life COPV vaccine induced skin SCC in 12 out of 500 treated beagles [5]. Several other studies carried

out using PCR and/or immunohistochemistry concluded that 5 to 30% of canine SCC harbour PV antigens or nucleic acids [13] Goldschmidt detected additionally a novel virus, namely CPV2, in numerous warts and SCC of several immuno-compromised Beagles [14].

Skin SCC in cats

Two different studies have demonstrated the presence PV DNA in feline BISC and invasive SCC [15]. One of these studies also suggested that the positive samples were infected by PV of great genetic diversity. One of this virus has been additionally recently described [10]. IHC studies have confirmed the causal association between virus infection and development of the disease [12]

PV-induced cell transformation

Three early PV genes code for the transforming proteins E5, E6 and E7 but not all PVs are able to immortalize and transform epithelial cells. Further, substantial differences exist in the transforming properties of oncoproteins.

E5 proteins are small polypeptides with transforming properties. They are the major oncoprotein of bovine PV [16]. They induce transformation by enzymatic functions, such as activation of several kinases [16]. The open reading frame coding for E5 is often deleted in cervical carcinomas in women, indicating that this gene does not play an essential role in maintaining the oncogenic phenotype in such cancers [17]. The role of E5 in the development of skin cancer in humans, dogs and cats is unknown.

As they play a major role in the development of cervical cancer in women, transforming and immortalizing properties of E6 and E7 have been extensively studied. The development of such cancer has been linked to persistent infections with high-risk HPV and is generally preceded by a lengthy latency period [18]. As low-risk HPV genome usually remains episomic, genomic integration is one of the key-events of the carcinogenesis induced by high-risk HPV[19]. After integration, E6 and E7 expression is maintained but regulatory E2 is deleted. The absence of E2 expression provides a growth advantage to affected cells [19]. E7 protein interacts with retinoblastoma tumor suppressor protein (pRb) and related pocket proteins such as p107 or p130.

These proteins regulate the activities of transcription factors (E2F family) that control the cell cycle. Additionally, E7 alters cyclin expression (p21 ^{CIP1}, p27 ^{KIP1}). All in all, E7 contributes to creating and maintaining a replication competent cellular milieu [18, 19].

On the other hand, the E6 of high-risk mucosal HPV bind the p53 tumor suppressor protein as part of a complex with the ubiquitin ligase, E6AP, leading to the rapid turnover of p53 [20]. As p53 is a potent inhibitor of cell growth, arresting the cell cycle at several points, and under some circumstances, activating the apoptotic machinery, its destruction is a major event in the transformation and immortalization [21].

Additionally, E6 and E7 induce cell immortalization through activation of telomerase activity and genomic instability through induction of centrosome abnormalities [19].

Interestingly, the E6 of cutaneous HPV does not bind p53 and does not promote its degradation [22]. Furthermore, p53 is very often mutated in human skin cancers and these

mutations are characterized by a specific signature attributed to ultraviolet radiation [23]. E6, in turn, targets bak for degradation and inhibits UVR-induced apoptosis [22]. These findings emphasize that cutaneous HPV-E6 contribution to skin cancerization is markedly different from that of mucosal high-risk HPVs.

The association of HPV and genital cancer is now well established with many lines of evidence supporting the causative relationship [2]. On the other hand emerging evidence suggests that cutaneous HPVs, including EV-HPV are not able to induce keratinocyte immortalization [22]. Cooperation between UV light and HPV is probably mandatory for skin oncogenesis.

INVITED LECTURES ABSTRACTS

L09

Neural stem cells and gliomagenesis

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The WHO 2007 classified gliomas in different subgroups according to their presumtive origin: astrocytic, oligodendroglial and mixte and into different grade of malignancy.

Pilocytic astrocytomas (mostly grade I) are benign tumors whereas glioblastomas, the most malignant glioma are grade IV. Although GBM are mostly primary, some grade II astrocytoma might become anaplastic (III) or even more malignant (grade IV) leading to the so-called secondary GBM.

The recent evidence of isocyanate deshydrogenase 1 (IDH1) or IDH2 mutation in the subgroups of grade II, III and secondary glioblastomas but not in primary GBM suggest that the target cell is different.

Evidence for cancer stem cells in GBM has suggested that GBM might derive from the neoplastic transformation of neural stem cell. Glioblastoma cancer stem cell (GBM CSC) are defined by 1) the capacity to self renew, 2) the ability to initiate brain tumors under orthotopic implantation and 3) multipotency, that is the capacity to differentiate into cells with a neuronal, astrocytic or oligodendroglial phenotype. However, characterization of human GBM CSC remains a challenge, likely because different subgroups of GBM occur that might derive either from different neural stem cells or from similar cells that have acquired different genetic alterations. Although C133 was first reported as the "CSC" marker (1) further studies have also shown that CD133- cells also have CSC properties (2). Following this line we and others have found that cells expressing L1 CAM or the ganglioside A2B5 also have CSC properties (3-5). Moreover we have reported that three populations of cells exists in GBM : The $A2B5^{+}/CD133^{+}$ the A2B5/CD133- and the $A2B5^{-}/CD133^{-}$. Only cells expressing the ganglioside A2B5 have CSC properties. Besides, some authors have emphasized the relationship between GBM location and pattern of relapse in one hand (6) and CD133 expression in the other hand (7). Therefore GBM from different locations might contain distinctive subsets of CSC. In contrast to GBM, it is possible that grade I and grade II glioma also derives from progenitor cells that have not been fully characterized. However, we have recently reported that pilocytic astrocytomas might derive from radial glial cells and exhibit distinctive signature according to their location (8) whereas grade II gliomas might derive from glial precursor cells (9-10). At last, the reason why glioma in infants usually do not share with adult glioma the same genetic alteration such as IDH1/2 mutation or 1p19q co-deletion is not fully understood but might be related to differential susceptibility to genetic transformation according to age. Taken together these data suggest that glioma heterogeneity not only rely on distinctive genetic alterations but also from different cell origin among which neural stem cells and glial progenitors, that might differ according to location, play a major role.

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INVITED LECTURES ABSTRACTS

L10

Neoplastic pathology and cytology of lung

THE NEW TNM CLASSIFICATION AND ITS IMPLICATIONS ON MACRO- AND MICROSCOPIC ANALYSIS OF LUNG CANCER SPECIMENS

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While the first tumor, node, metastasis (TNM) stage grouping system was developed in the 1940s, only in 1986 did the American Joint Committee on Cancer (AJCC) and the Union Internationale Contre le Cancer (UICC) agree on an international system for staging malignant neoplasms. The 1997 revision, better known as the 6th edition, was based on 5319 *mostly surgical* cases from MD Anderson Hospital in Houston, Texas, USA dating back to 1975. The 6th edition also presented the first standardized regional lymph node station classification.

The 7th edition TNM system for lung cancer was introduced in late 2009 and formally replaced the 6th edition on January 1, 2010. Unlike prior revisions, this project was not undertaken by the UICC or AJCC, but rather by the International Association for the Study of Lung Cancer (IASLC) and is based on over 100,000 *worldwide* lung cancer cases treated by *all modalities* from 1990-2000.

This 7th edition has several major changes and several minor departures from the 6th edition regarding T, N, and M designations (see attached pdf and table 1). These changes impact lung cancer specimen processing. Awareness of all these changes is absolutely necessary for correct pathologic staging of lung cancer patients. The following comments only highlight several of the major revisions but are by no means complete.

The T designation is impacted by several changes. While the 6th edition separated carcinomas 3.0 cm or smaller from larger tumors, the 7th edition added new size cut-offs at 2, 5 and 7 cm which determine differing T designations. Tumors should be measured prior to fixation since formalin fixation is known to shrink tissue samples. One recent study noted that formalin fixation-induced tumor shrinkage caused stage shift from stage Ib to stage Ia in 10% of a 401 patient study cohort.

The 7th edition attempts to clarify visceral pleural invasion (VPI). While VPI remains T2 and parietal pleural invasion T3 in the 7th edition TNM, the definition of pleural invasion has been altered. Given the complex microanatomy of the visceral pleura, especially in instances where a subpleural carcinoma is embedded in fibroelastotic and/or desmoplastic stroma, the 7th edition simplifies pleural evaluation by stating that a carcinoma must invade beyond the major 'thick' elastic layer to warrant a T2 designation. This is conceptually an improvement over the 6th edition requirement of 'invasion of the lamina propria serosae.' (These rules still differ from the Japanese Lung Cancer Society classification, which upstages T based on both tumor size and VPI.) Whether this simplification along with a reminder to use elastic tissue stains will lead to reproducible visceral pleural staging remains to be seen.

Obviously one must submit entire pleural puckers and subpleural scars for microscopic assessment. If one is not certain about VPI, then one should follow the general TNM rules and assign the lower

category T. Parenthetically, the 7th edition staging system designates, despite the absence of clear data, any carcinoma invading into an ipsilateral lobe, i.e., across a fissure, T2a.

The 7th edition also dramatically revised the TNM approach to multiple tumor nodules. First, the confusing term 'satellite nodules' has been replaced with the more reasonable descriptor 'additional tumor nodules.' Yet the pathologist must be aware that these lesions *must* be recognized grossly and that incidental microscopic additional tumor nodules *do not* impact the TNM designation. Grossly identified 'additional tumor nodules' in the major tumor-bearing lobe require a T3 assignment, while separate tumor nodule(s) in a different ipsilateral lobe require a T4 assignment. Separate tumor nodule(s) in a contralateral lobe are staged M1a.

Historically, two competing lymph node maps describe regional lymph nodes extending from the supraclavicular region to the diaphragm. Nomenclature for the anatomical location of the lymph nodes differs between the Naruke—Japan Lung Cancer Society map and the Mountain-Dresler modification of the American Thoracic Society North American-European system. The 7th edition TNM features an amalgamated map that that maintains fourteen nodal groups and proposes grouping N1 lymph nodes into Hilar/Interlobular and Peripheral zones, and N2 nodes into five separate zones; Supraclavicular, Upper, AP, Subcarinal and Lower. This 'zone' concept requires validation. While there is evidence to support the recommendation that lymph node sampling should include at least six lymph node stations, three of which should be mediastinal with subcarinal (level VII) lymph nodes included, as long as a single lymph node is sampled and is free of carcinoma, a designation of N0 is permitted.

The 7th edition TNM also acknowledges the possibility of nodal and metastatic isolated tumor cells (ITC), but the finding does not alter the N or M designations. Single tumor cells or small clusters 0.2 mm or smaller in greatest dimension are usually detected by immunohistochemistry, flow cytometry, or molecular methods. These ancillary tests are required at the present time and single histological sections of all submitted lymph nodes suffice for patient care. pN0 means that that no regional lymph node metastasis are noted histologically, and no examination for ITC was performed, while pN0(i-) indicates that no regional lymph node metastasis are noted histologically, and confirmed morphologically with immunohistochemistry. pN0(i+), indicates that no regional lymph node metastasis are noted histologically, but morphologically identified with immunohistochemistry.

As alluded to above, the IASLC has also altered and subdivided the M category. Whereas the 6th edition considered contralateral lung metastases and distant metastases M1 disease, the 7th edition divides M into lung/pleural metastasis (M1a) and distant metastasis (M1b). The M1a category consists of the contralateral lung additional tumor nodules, pleural nodules and malignant or pericardial effusions. The malignant effusion designation was a T4 in the 6th edition.

Beyond standard TNM issues, one should note that for the first time in lung TNM

history, carcinoid tumors resection reports require pathologic TNM (pTNM) staging. This development is worthy since it reinforces to our clinical colleagues that carcinoid tumors are malignant neoplasms. However, the decision to include the tumors in the pTNM classification does not mean that the stage groupings are infallible. In fact, the authors responsible for the inclusion of these tumors into the 7th edition enumerate many shortcomings in their research and essentially suggest the inclusion of carcinoid tumors as a data collection tool.

Briefly, the 392 collected surgical cases were tested against 1437 National Cancer Institute Surveillance Epidemiology and End Results (NCI-SEER) surgical cases. Clinical staging was not available. The pathologic distinction between typical and atypical carcinoid tumor was not available and cancer-specific cause of death were not known. Survival differences were noted for stage I versus stage II versus stage III/IV carcinoid tumors but not for the subcategories IA versus IV or IIA versus IIB. However, many issues remain unresolved including the validity of this system for typical versus atypical carcinoid tumors, the validity of staging multicentric carcinoid tumors and the validity of size cutoffs in the determination of T value.

The 7th edition is also far-reaching in its strict requirement that cancer centers TNM stage small cell carcinoma (SCLC) cases. The 1950's Veteran's Administration Lung Study Group (VALSG) 'limited (LD)' versus 'extensive (ED)' clinical staging system for SCLC was revised in 1989 by the IASLC. While easy to apply, this schema does not adequately assess different prognostic groups among the one-third of SCLC patients with LD, and thus does not allow for differing radiotherapy fields for this heterogeneous patient population. Clinical data from 8088 patients with SCLC diagnosed and treated between 1990 and 2000 indicate that individuals with clinical T1 disease had significantly better survival than those with T2 disease and all other T categories. And those with clinical N0 or N1 tumors had significantly better survival than those with N2 or N3 tumors, especially N1 versus N2. However, the ability of the TNM system to discern prognostic significance between ipsilateral and contralateral supraclavicular lymph node involvement is unknown. Currently both regions fall within the LD designation.

Whether one should apply a complex system to a carcinoma where less than 5% of patients are eligible for *pathologic* surgical staging is debatable; however, recent data from 349 surgically staged SCLC cases are provocative. Pathologic TNM staging of SCLC stratifies different prognostic groups according to stage. In addition, aggressive surgical staging, including mediastinal lymph node sampling, downstages up to 30% of patients with clinical stage III disease.

While the abovementioned changes require time to get acquainted with, note that the Prospective Lung Cancer Staging Project is underway and will likely result in yet a new and improved 8th edition TMN by the end of this decade!

- The 7th edition TNM is required for the classification of NSCLC, SCLC and carcinoid tumors
- The T classification has been refined
  - T1 is subclassified into T1a (<= 2 cm) and T1b (>2-3 cm)
  - T2 is subclassified into T2a (>3-5 cm) and T2b (>5-7 cm)
  - T2 (>7 cm) is reclassified as T3
  - Multiple tumor nodules in the same lobe are reclassified from T4 to T3
  - Multiple tumor nodules in the same lung but a different lobe are reclassified from M1 to T4
- The N classification is unchanged but a new international lymph node map defining the anatomical boundaries for lymph node stations is presented
- The M classification has been refined
  - M1 is subdivided into M1a and M1b
  - Malignant pleural and pericardial effusions are reclassified from T4 to M1a
  - M1b designates distant metastases

INVITED LECTURES ABSTRACTS

L11

Leptospirosis in man and animal

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Leptospira are long helical spirochetes that thrive shortly in a moderately warm, watery environment and which use mainly animal reservoirs such as all sorts of rodents. Men and other mammals can become infected by contact with infected animal tissues, urine or urine-contaminated water. Mainly the strains or serovars of *Leptospira interogans*, which are divided into more then 20 serogroups, can cause fever-like symptoms, hepatic disease of kidney failure.

The talk will address and present some observational data of the occurrence of Leptospirosis in Belgium. Data were gathered in the Belgian veterinary diagnostic laboratory (CODA-CERVA). In 2000-2001, low-titre leptospirosis mainly occurred in cattle (serovars Sejroe and Grippotyphosa) and pigs (serovars Pomona and Australis). At the same time, the most common found serovars in dogs and horses were Icterohemorrhagiae and Grippotyphosa. Since 2002, seropositivity in cattle, pigs and companion animals became very rare and dropped to less then 1% of the suspected cases. However, since 2006, a sudden increase of clinical cases was observed in dogs and horses. In the case of serovar Australis infections in dogs, acute kidney failure was prominently present. In the case of Pyrogenes, hepatic disease dominated and most animals recovered. Some cases will be presented

INVITED LECTURES ABSTRACTS

L12

Bowen disease versus Bowenoide in situ Carcinoma

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Actinic keratosis (AK), Bowen's disease (BD), Bowenoid papulosis, erythrodysplasia of Queyrat and epidermodsyplasia verruciformis are regarded as various forms of squamous cell carcinoma *in situ* in man [1]. Two forms, AK and Bowenoid in situ carcinoma (BISC) have been described in feline [2-4] [5]. Feline AK are plaque-like to papillated solitary or symmetrical scaly lesions that occur mostly on the pinnae, nose and eyelids of white cats [3]. BISC lesions are often multifocal, crusted plaques that occur in any location, including dark pigmented and non sun-exposed haired areas [2-4]. Histologically, BISC lesions are characterized by irregular epidermal hyperplasia and dysplasia without invasion through the basement membrane. Hair follicle infundibula and isthmi may be involved in the hyperplastic process [2-5]. AK lesions are usually less hyperplastic and hair follicles are less deeply affected. Nonetheless the histological differences are not always sufficient to distinguish both diseases and the pathologist may render a differential diagnosis. The final diagnosis then has to be made based on clinical features such as the affected area and the color of the skin/hair coat [3, 5].

The main goal of this presentation is to provide clues narrowing down the differential [6].

	BISC	AK
1 or 4X	• Basement membrane intact • Discret plaque • Moderate to severe irregular epidermal hyperplasia • Moderate to marked hyperkeratosis (ortho, para), sero cellular crust • full epidermal thickness dysplasia • Follicular infundibula similarly involved • Formation of broad rete ridges	• Basement membrane intact • Skin at margins is thickened • Mild to moderate epidermal hyperplasia • Moderate to severe laminated or compact hyperkeratosis or parakeratosis • Dysplasia more in basal and lower spinous cells • Only superficial involvement of infundibular epithelium • Possible irregular rete ridges formation of angular profiles not perpendicular to the skin surface

10X	• Cells resemble basal cells • Marked loss of nuclear polarity • wind blow pattern • Disruption of normal epithelial stratification • May have multinucleated cells • Nest formation	• Cells atypical but still resemble spinous cells • Mild to moderate architectural distortion • Loss of polarity in basal and spinous layers • May have multinucleated cells
40 X	• More basophilic cytoplasm • Nuclear pleomorphisme, ovoide hyperchromatic to large • viral cytopathology : koilocytes, large round keratohyaline granules • apoptotic cells as AK+/- • Mitotic activity above memb basale	• Mild to moderate nuclear atypia – Nuclear enlargment pleomorphie – Nucleolar prominence – Occasionnal hyperchromasia • Mitotic activity above the memb basal • Scattered apoptotic cells
Within the derm.	• Vascular dilatation nonspecific perivascular inflammation	• Dermal inflammation collagen degeneration • Fibrosis • Some time look like lichenoide AK as human

The differential is sometime not possible as in case of AK with severe dysplasia. The following criteria were then the most useful: the lesion's borders, the more basophilic (basaloid) or eosinophilic (keratinocytic) appearance of the cytoplasm. The presence of pigmentation, the solar changes within the dermis (although in BISC within white exposed areas those criteria's are overlapping). But even then, it is the clinic and the identification of P53 mutation or Papilloma virus antigen that allow the final diagnose as DR Favrot as developed.

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INVITED LECTURES ABSTRACTS

L13

Hantavirus infections in Europe: from virus CARRIERS TO major health problem.

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OBJECTIVES : In Europe, hantavirus disease or Hemorrhagic Fever with Renal Syndrome (HFRS) is an endemic zoonosis that affects tens of thousands of individuals. During the past decade the impact of hantavirus infection on the health status of companion and production animals was studied. The causative agents are viruses of the genus Hantavirus, family *Bunyaviridae*, rodents and insectivores act as carriers. In all European countries there is a seroprevalence for hantaviruses in the general population but not all countries also report cases for various reasons. Here we give an overview of the hantavirus situation in Europe.

METHODS: A survey was conducted on request of the European Network for diagnostics of Imported Viral diseases (ENIVD), a European Centre for Disease Prevention and Control (ECDC) network. National Reference Laboratories of EU member states were invited to complete a questionnaire. Data from this questionnaire were statistically analyzed.

RESULTS: Analysis of the data provided insight in the various national regulations, the geographical boundaries of co-circulation of Puumala (PUUV) and Dobrava (DOBV) virus in Europe, incidence rates, clinical presentation and epidemiological patterns in different European Union countries. These results will be discussed in detail.

CONCLUSION: The understanding and recognition of hantavirus infections has greatly improved in Europe over the past few decades. In the past decade both amplitude and magnitude of hantavirus outbreaks has been increasing. This could partly be due to increased awareness, better diagnostics and intensified research into reservoir species but human invasion of previously undisturbed habitats (by building houses, factories, etc) and changing climatic conditions (warmer winters, increased frequency of mast events) certainly contributes to the problem. Despite the progress, many questions remain without answer. Further investigations are necessary to elucidate these questions.

INVITED LECTURES ABSTRACTS

L14

Lung tumours, and in particular pre-neoplastic lesions

K. Kerr

The WHO classification is a complex system accounting for a very wide range of tumours which arise in the lung. This presentation will consider primary epithelial tumours in the lung, with a focus upon their origin from pre-invasive lesions. Interest in pre-invasive lesions has risen after a clear classification of precursors was introduced in the WHO classification in 1999, as the molecular biology of these lesions became better understood and with a resurgence of interest in lung cancer screening. For some types of lung cancer, the likely precursor lesion is not understood. There are probably pathways of lung cancer development, and precursor lesions still to be described.

The main groups of primary lung carcinoma are:

Small cell carcinoma

Squamous cell carcinoma

Adenocarcinoma

Large cell carcinoma

Sarcomatoid carcinoma

Adenosquamous carcinoma

Carcinoid tumour

Salivary-type carcinomas

Small cell carcinoma (SCLC)

A highly malignant morphologically undifferentiated carcinoma showing evidence of neuroendocrine differentiation at a molecular and ultrastructural level. Characteristic nuclear morphology (granular, featureless), nuclear moulding, common smear artefacts and occasional organoid architecture. The vast majority of these tumours are central bronchogenic lesions. This tumour pattern may occur in association with non-small cell carcinoma patterns, in which case the diagnosis remains SCLC of combined type. SCLC may take an origin in squamous dysplasia / carcinoma in situ (SD/CIS) but this is not certain.

Squamous cell carcinoma

A malignant epithelial tumour which, by definition, shows evidence of squamous differentiation in the form of cell keratinisation and/or intercellular bridges. NO OTHER FEATURE defines squamous differentiation. These lesions are also typically central bronchogenic tumours but peripheral squamous cell carcinomas also occur and may be on the increase. Many probably arise in SD/CIS.

Variants of squamous cell carcinoma include basaloid, small cell, clear cell and papillary forms. Basaloid is probably the most important variant as it may indicate a poorer prognosis.

Adenocarcinoma

A complex and diverse group of carcinomas, unified by evidence of mucin production and / or 'glandular' differentiation. Most adenocarcinomas show a mixture of patterns on histological examination: acinar, papillary, micropapillary, solid (with mucin) and

bronchioloalveolar patterns are described. Other patterns such as signet-ring, clear cell and cribriform tumour exist. Most arise peripherally, from the bronchioloalveolar epithelium (the terminal respiratory unit – TRU) but some undoubtedly arise in central bronchi and probably also from smaller bronchi and bronchioles. TRU-type carcinomas most likely take their origin from atypical adenomatous hyperplasia (AAH). Pure localized non-mucinous tumours showing a pure lepidic growth pattern and no evidence of invasion are currently classified in WHO as bronchioloalveolar carcinoma (BAC) but are better considered Adenocarcinoma in situ (AIS). A progression from AAH to AIS to invasive adenocarcinoma is proposed.

Large cell carcinoma

This is a heterogeneous collection of tumours:

Large cell carcinoma NOS is a diagnosis of exclusion, the tumour lacking LIGHT MICROSCOPIC H&E evidence of differentiation and comprising generally large nucleolated malignant cells. May be peripheral or central.

Basaloid carcinoma is a rather controversial entity which morphologically resembles basal cell carcinoma in other organs. It appears to be biologically aggressive.

Specific precursor lesions for these tumours are not recognized. Some basaloid carcinomas may co-exist with SD/CIS. It is possible that these two forms of large cell carcinoma may also represent de-differentiated progression of squamous or adenocarcinomas.

Large cell neuroendocrine carcinoma (LCNEC) comprises large nucleolated cells with abundant, often eosinophilic cytoplasm and prominent organoid architecture. In addition to the 'neuroendocrine' morphology, neuroendocrine differentiation should be proven using immunohistochemistry or electron microscopy. There is no known pre-invasive precursor for LCNEC. LCNEC may co-exist in the same lesion with SCLC, squamous cell carcinoma but most often with adenocarcinoma.

Other types in the Large Cell category are clear cell carcinoma, lymphoepithelioma-like carcinoma and large cell carcinoma with rhabdoid features.

Sarcomatoid carcinoma

Sarcomatoid carcinomas may exist in pure form or combined with other patterns of non-small cell carcinoma. These are aggressive tumours of central or peripheral location. Sarcomatoid histology includes pleomorphic, spindle cell and giant cell carcinoma. There is no known precursor lesion but, again, they could represent dedifferentiation of differentiated adeno or squamous cell carcinomas.

Adenosquamous carcinoma

These are exceptionally rare tumours when defined strictly as lesions comprising at least 10% of each of differentiated squamous cell and adenocarcinoma. They appear to predominate in the lung periphery. No recognized precursor lesion.

Carcinoid tumour

Low grade malignancy of neuroendocrine cells, these are invasive lesions which may metastasise. Typical and atypical forms exist, the latter defined by the presence of necrosis and/or more than 2 mitoses per 2 mm^2 of tumour. Most arise centrally from large bronchi and show usually an insular/trabecular pattern. Peripheral carcinoids usually have a spindle cell

morphology. Central carcinoids have no known precursor. Peripheral spindle cell lesions are sometimes associated with diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH), a recognized carcinoid precursor in the WHO classification.

Salivary-type carcinomas

Adenoid cystic and mucoepidermoid carcinomas are rare in the lung, predominate in the trachea and main bronchi, probably originate in the bronchial sero-mucous glands but an actual precursor lesion is not known.

Precursor lesions

Squamous Dysplasia / Carcinoma in situ

This is the archetypal precursor of bronchogenic carcinoma, mainly squamous cell carcinoma but has been associated with small cell and basaloid carcinomas. The WHO classification allows for three grades of dysplasia (mild, moderate, severe) as well as CIS. It is debatable whether basal cell hyperplasia or squamous metaplasia are precursors for SD; probably both are.

Strongly associated with smoking, it may also be seen in the lung periphery in some cases of lung fibrosis and may account for some of the cases of lung cancer arising in this clinical setting. Grading SD is difficult. SD/CIS is generally invisible at bronchoscopy, may be highlighted using autofluorescence bronchoscopy (AFB) and is generally an incidental finding in biopsy samples unless AFB is used.

Atypical adenomatous hyperplasia (AAH)

A putative precursor of AIS/BAC (see above), these small mm-sized lesions occur in the centriacinar regions of the lung parenchyma, are usually incidental histological findings, show proliferation of bronchioloalveolar cells around adjacent alveolar walls and higher grade more atypical and cellular lesions merge with AIS/BAC in a spectrum of change.

AIS/BAC is the immediate precursor of those TRU-type peripheral invasive adenocarcinomas. Here architectural collapse and fibroplasia herald stromal invasion and the development of invasive adenocarcinoma.

AAH and AIS are most often found in lungs resected for adenocarcinoma, supporting the belief that they are precursor lesions.

Quite distinct from AAH is the atypical alveolar lining cell proliferation seen in lung fibrosis which may account for some of the adenocarcinomas which are seen in excess in patients with these underlying conditions.

Diffuse Idiopathic Pulmonary Neuroendocrine Cell hyperplasia (DIPNECH)

This is a rare disease in which peripheral airway neuroendocrine cells (NEC) show multifocal proliferation in the TRU-zones and larger bronchioles. Linear proliferations of basal NEC, neuroendocrine cell nodules or bodies, carcinoid tumourlets and spindle cell carcinoid tumours may all be found. Patients may present with the tumour, or with an asthma-like syndrome. Some cases are incidental. Carcinoid tumours may be multiple and may be atypical.

INVITED LECTURES ABSTRACTS

There are many issues relating to the origin of lung cancers which remain unresolved. A significant proportion of lung adenocarcinomas probably do not arise from the AAH-AIS precursor in the TRU, the origin of small cell carcinomas remains obscure and some patterns of mucinous adenocarcinoma also ask major questions of current classifications. There are uncommon pre-existing lung diseases / lesions which rarely transform to malignancy. Some lung cancers may arise *de novo*, without any morphologically recognizable precursor lesion.

INVITED LECTURES ABSTRACTS

L15

Pathobiology of Hodgkin lymphoma

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Hodgkin lymphoma (HL) is subdivided into a classical and a nodular lymphocyte predominant subtype. Classical HL (cHL) accounts for about 95% of cases, the remaining 5% are nodular lymphocyte predominant HL (NLPHL). In both types of HL, the tumour cells, the Hodgkin and Reed/Sternberg (HRS) cells in cHL and the LP cells in NLPHL, usually represent <1% of the lymph node cellularity. LP cells show a gene expression pattern and molecular features that indicate a derivation from germinal centre B cells. HRS cells have largely lost the B cell gene expression pattern, but analysis of their rearranged immunoglobulin V genes shows a B cell origin also of these cells; HRS cells are likely transformed "crippled" germinal centre B cells that normaly would have undergone apoptosis (1).

To identify deregulated genes in HRS and LP cells and reveal their relatedness to each other and to other normal and malignant B cells, we generated Affymetrix gene expression profiles from 1000-2000 laser-microdissected primary HRS and LP cells and various other B cell lymphomas, normal B-cell subsets and HL cell lines. The analysis of profiles from NLPHL indicated a relationship of LP cells to and/or origin from germinal center B cells at transition to memory B cells. LP cells show a partial loss of their B cell phenotype and are characterized by constitutive NF- B activity (2).

Constitutive nuclear activity of NF- B represents a key feature in the pathogenesis of cHL. We sequenced the complete coding region of TNFAIP3, encoding for A20, an inhibitor of NF- B, from cHL cell lines and HRS cells of primary biopsies of cHL. We detected somatic mutations in 16 of 36 cHL, mostly in Epstein-Barr virus (EBV)-negative cases (3). Reconstitution of wildtype *TNFAIP3* in A20-deficient cHL cell lines resulted in a significant decrease in transcripts of NF- B target genes and caused cytotoxicity. Thus, *TNFAIP3 (A20)* functions as a tumor suppressor gene in cHL. The significantly higher frequency of *TNFAIP3* mutations in EBV-negative than EBV-positive cHL suggests complementing functions of *TNFAIP3* inactivation and EBV infection in cHL pathogenesis. Notably, mutations in TNFAIP3 (and also NFKBIA) are uncommon in LP cells of NLPHL (4).

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INVITED LECTURES ABSTRACTS

L16

Molecular markers in lung carcinoma

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INTRODUCTION

Lung cancer is the leading cause of cancer-related death in men and women worldwide, with an overall survival at five years less than 15%, and iimprovement the survival rate of patients with lung cancer requires the understanding of molecular events leading to lung cancer, in order to identify genetic markers implicated in tumoral progression, to improve earlier detection of lung cancer in high- risk patients, and to develop novel targeted therapeutic strategies including chemoprevention.

Nearly 80 to 90% of lung cancer is related to cigarette smoking, but the incidence of adenocarcinoma is changing for the last ten years, the increasing number of cases being possibly related to modern cigarettes containing certain carcinogens. However not all smokers will develop a lung cancer, suggesting additional epidemiological factors, such as genetic individual susceptibility.

Lung carcinogenesis is a multifocal ("field cancerization") and multi -step process, resulting from the sequential accumulation of molecular and genetic/epigenetic abnormalities. These abnormalities lead to the activation of growth promoting oncogenes and the inactivation of tumor suppressor genes, favouring proliferation and resistance to apoptosis. In that respect, Loss of Heterozygosity (LOH) or homozygous deletion are frequently observed at tumor suppressor gene loci. Alternatively, activation of oncogenes may be due to genetic modification, including mutation, amplification or chromosomal rearrangement, or epigenetic modification such as hyperexpression. Dysregulation of DNA repair, signal transduction, and the cell cycle represent potential drug targets and biomarkers.

Genetic susceptibility

Among the 20 carcinogens contained in tobacco smoke and strongly related to lung cancer development, polycyclic aromatic hydrocarbons and nicotine derived nitrosaminoketone are responsible for DNA adduct formation. This adduct formation is caused by metabolic activation of these carcinogens by P450 cytochrome enzymes, encoded by CYP family genes, and a high activity of P450 cytochrome is a risk factor of lung cancer. In the same way, a null genotype of an enzyme responsible for detoxification, the glutathione S-transferase (GST), favors lung cancer. In addition, familial gene susceptibility locus has been found at 6q23-q25 (RGS17 gene) and according to a genome-wide association study, SNP linked to lung cancer are located at 15q25.1 encoding for nicotinic acetylcholine receptor pathway alpha subunits 3 and 5 (CHRNA3 and 5 genes), and responsible for nicotine addiction and a higher risk of lung cancer.

DNA damage repair: NER pathway

Genes implicated in DNA repair and nucleotide excision (NER system) are considered as drug resistance markers, ERCC1 and RRM1 having being reported in lung cancer as influencing poorly the sensitivity to cisplatin and gemcitabine. ERCC1 is a rate-limiting protein in the NER pathway, which recognizes and removes platinum adducts and repairs interstrand DNA cross-links. Cisplatine naive patients have a longer survival when they have a higher ERCC1 expression than those with a low ERCC1 expression (by qPCR or immunohistochemistry). In contrast, overall survival was significantly lengthened by chemotherapy for patients with a low ERCC1 expression.

RRM1 is the regulatory component of ribonucleotide reductase, involved in DNA synthesis and repair. It also suppresses cell migration and metastasis by inducing PTEN expression. RRM1 is the predominant target of the nucleoside analogue Gemcitabine. Median disease-free survival for untreated patients with tumors with a low RRM1 expression is lower (54 mo) than those with a high RRM1 expression (120 mo), but longer if patients are treated with gemcitabine.

Tyrosine Kinase signaling pathways

1. EGFR family

The EGFR (Epidermal Growth Factor) family are transmembrane tyrosine kinase receptors and are composed of EGFR (HER1 or ERBB1), HER2 (EGFR2 or ERBB2/Neu), HER3 (EGFR3 or ERBB3) and HER4 (EGFR4 or ERBB4). Mutation or hyperexpression of these receptors in lung cancer are responsible for their oncogenic activation, favoring proliferation, differentiation, apoptosis and angiogenesis. When linked to their ligands, receptors form auto or heterodimers, leading to their autophosphorylation, and activation of intracellular cascades implicating Ras, Raf, MAP/kinase, SRC, STAT, and the PI3-kinase/AKT.

Signaling pathway of EGF receptors seems to be mainly impaired in adenocarcinoma, with a typical autocrine loop described in alveolar carcinogenesis. Hyperexpression of EGFR1 and HER2 is reported in 70 and 30% of NSCLC, respectively, either in relation with a gene amplification or an increased transcription. There are several drugs targeting EGFR and HER2, including small molecule TK inhibitors Gefitinib (Iressa), Erlotinib (Tarceva) and the monoclonal antibodies cetuximab (Erbitux) and trastuzumab (Herceptin, targeting HER2). In 2004, mutations of TK domain of EGFR have been described, corresponding predominantly in deletion in exon 19 (44%) and missense mutations (L858R) in exon 21 (41%); interestingly, these mutations correlate with sensitivity to TK inhibitors. EGFR mutations are more frequent in women, from Asia, and non-smokers. However, a second type of mutation contributing to an acquired resistance to TK inhibitors was recently discovered (T790M, exon 20 insertion). Of note, hyperexpression of EGFR detected by immunhistochemistry has no to date any prognostic value but mutations or gene amplification (i.e. increased number of gene copies detected by FISH) appear to be the best predictors of TKI response.

2. Ras family

Ras family of genes encodes 21kDa proteins binding GTP to form ras-GTP complex, transducing proliferation signals. Their activation in ras-GTP activates transcription factors

such as C-fos, C-jun, C-myc and DNA synthesis. Activating ras mutations are mostly identified at codon 12 of the K-ras gene, more rarely at codons 13 and 61, and are induced by tobacco carcinogens like benzo-a-pyrene and nitrosamine. Ras mutations are detected more frequently in adenocarcinomas and large cell lung carcinomas.

The pronostic value of K-ras mutations is still controversy but a meta-analysis has suggested a shorter survival for patients with mutation. This mutation could be responsible for resistance to cytotoxic drug (cisplatin) or to TK inhibitors (Gefitinib, Erlotinib). Interestingly, K-ras mutation and EGFR mutations are said to be mutually exclusive.

3. PI3 kinase/ AKT

PI3 kinases are lipid kinases regulating proliferation, growth, apoptosis, and cytoskeletal arrangement. These proteins are constitutively activated in most lung cancers. PI3KCA encoding for a 110 kDa catalytic subunit of PI3K is mutated in 3% of NSCLC. PI3KCA mutations are found in 2.5% of NSCLC and gains of chromosome 3q22-q26 are detected in 33% of squamous cell carcinoma, while PI3KCA over-expression, related to gene amplification at 3q26, in found in 40% of SCC . it is associated with an increased PI3KCA activity, a PI3K inhibitor resistance and AKT, a downstream effecter of PI3 kinases, activation. AKT is constitutively activated in 16/17 NSCLC cell lines with oncogenic effects. PTEN is a negative regulator of AKT, and its loss or reduced expression (by hypermethylation or 10q23 deletion) is observed in nearly 10% of lung cancers.

4. ALK fusion proteins

A recurrent gene fusion between Echinoderm Microtubule-associated protein- Like 4 (EML4) and the anaplastic lymphoma kinase (ALK) gene occurs in nearly 7% of NSCLC, resulting in an AKL fusion protein. It is preferentially observed in adenocarcinoma of solid or acinar architecture, with signet ring cells and TTF1 positive. Patients are younger males, non- or light smokers (< 10 py), and with advanced stages. This gene fusion is mutually exclusive with EGFR /Ras mutations, and when present there is no benefit with TKI but a partial response with MET/ALK inhibitors.

Tumor suppressor genes

In contrast with oncogenes, tumor suppressor gene inactivation may be due to mutation, loss of chromosomal material (one or two alleles) or epigenetic phenomenon such as methylation. One of the most common chromosomal abnormalities in lung cancer is allelic deletions or LOH (loss of heterozygosity) at sites where tumor suppressor genes map: 3p (FHIT and others), 9p (9p21 for $p16^{INK4}$, p15 ^{INK4B} and $p19^{ARF}$), 17p (17p13 for p53 gene and others), 13q (13q14 for retinoblastoma gene and others). 3p and 9p losses have been associated with smoking and are recognized as early events of lung carcinogenesis. They remain detectable many years after smoking cessation. The loss of 17p13 is less common, suggesting that p53 alterations are rather late event or that they proceed from other mechanisms such as mutation. The frequency and the number of chromosomal abnormalities parallel the phenotypic progression from premalignant lesions to invasive cancer. Deletions affecting 3p, 5q, 8p, 9p, 17p and 18q chromosomal regions are among the common changes in lung cancer.

Hypermethylation of tumor suppressor gene promoters is known to be an important epigenetic mechanism for TSG inactivation, and could serve as early lung cancer detection marker. However, this process of hypermethylation is frequently observed diffusely in smokers' bronchial tree ($P16^{INK4}$, RAR beta, FHIT, MGMT, RASSF1, DAP kinase, APC, and CDH1 (E cadherin)), whereas genes only methylated in malignant cells remain very rare. Nevertheless, hypermethylation of a high number of genes seems to be correlated with a higher risk of lung cancer and could be observed 3 years before the occurrence of a detectable tumor.

1. P53

P53 is a tumor suppressor gene called the « guardian of genome », which acts as transcription factor for G1 arrest control and apoptosis. It reduces Rb phosphorylation and induces a stop at the G1-S checkpoint to allow DNA repair or to drive cells to apoptosis mediated by Bax/Bcl2. Its properties are abrogated as a result of mutation or inhibition of p53 pathway alterations. About 70% of lung cancers present a p53 mutation, which induces its abnormal stabilization. Mutations are detected in 70 to 100% of small cell lung cancers and in 45 to 75% of non-small cell lung cancers. In preinvasive lesions, a P53 aberrant expression is found from the level of mild dysplasia (25%) to that of CIS (75%), and along with K- ras mutation, P53 mutation in one of the most powerful tool for early lung cancer diagnosis and detection. The most common p53 mutation is a GC to TA transversion. A strong correlation is observed between the frequency of these mutations and the global duration of tobacco exposure. When stabilized, the P53 protein is no longer able to link with Mdm2, which normally degrades P53 into the proteasome. In addition, the downstream apoptotic pathways, implicating Bax/Bcl2 and Fas/ Fas Ligand, can also be altered in the absence of p53 mutations.

P53 is an effector of $P14^{ARF}$ and Mdm2, and $P14^{ARF}$ is able to link Mdm2 and to sequestrate it into the nucleolus in order to prevent P53 degradation. $P14^{ARF}$ and Mdm2 are alternatively altered and are responsible for a functional P53 inactivation even when p53 is not mutated. $P14^{ARF}$ is considered as a tumor suppressor gene responding to oncogenic stimuli (Ras, Myc, and E2F1). $P14^{ARF}$ is lost in 40 to 60% of SCLC, often concomitantly with P53 mutation. $P14^{ARF}$ is responsible for G2 arrest and apoptosis related to DNA damage response mediated by ATM and CHK2. Hyperexpression of Mdm2 is observed in 30% of NSCLC and SCLC and this overexpression is exclusive with $P14^{ARF}$ loss, reported in 40% of adenocarcinoma and SCLC.

2. Rb pathway

Rb protein is the main effector of G1 arrest mediated by p53 in case of DNA damage or oncogenic stress. Rb protein expression is lost in 80% of SCLC but only in 15% of NSCLC and never in preinvasive lesions. In contrast, Rb inactivation through deregulation of its phosphorylation is common in NSCLC. Two mechanisms are responsible for this deregulation: the loss of the CDK inhibitor $p16^{INK4}$, which negatively controls the CDK-cyclin activity, and the overexpression of cyclin D1. Inactivation of $p16^{INK4}$ in NSCLC is mainly caused by exon 1 or 2 mutation (15%), homozygous deletions (30 to 40%) or promoter methylation (30 to 40%), and there is a strict inverse relation between Rb and P16 expression. Hypermethylation of p16 can be detected in bronchial epithelium from chronic smokers with a high risk, suggesting inactivation of p16 occurs early in lung tumorogenesis. P16 methylation can be also detected with a very high sensitivity (one allele methylated detected over 10^4 normal unmethylated alleles) in exfoliated cells, and thus represents a useful tool for early detection of lung cancer.

More recently the role of cyclins E, D1 and B1 as important oncogenes in lung cancer was also highlighted. Cyclin D1 and/or cyclin E overexpression is responsible for deregulation of Rb phosphorylation in about 50% of lung carcinoma and is an early event in preinvasive process as it can be detected by immunohistochemistry in half of dysplasia increasing in frequency with their grade.

Telomerase

Telomerase is the key enzyme stabilizing the telomeres, defined as highly complex terminal chromosome structures, which correct function is crucial for normal cell survival.. Telomerase is preferentially expressed in tumor cells with short telomeres and is not expressed in most somatic cells, which usually have longer telomeres. Telomerase is expressed in 80-85% of non small-cell lung cancers and in almost all small-cell lung cancers. Telomerase activity is detected in precancerous lesions of the lung, reflecting the early involvement of the molecule in lung tumorogenesis. Telomerase is a prognostic factor in early-stage non-small cell lung cancer. Furthermore, telomerase activity has been correlated with cell proliferation, TNM tumor stage and node invasion.

CONCLUSION

Incidence and mortality associated with lung cancers have not been significantly modified since more than 25 years, despite introduction of new cytotoxic drugs and development of multidisciplinary approaches combining surgery, chemotherapy and radiotherapy. This points out how much chemoprevention approaches, to prevent airways carcinogenic process, are necessary. Complete characterization of molecular determinants of lung or head and neck carcinogenesis is essential to allow rational and targeted development of chemopreventive agents.

L17

Neuroendocrine tumors of the lung

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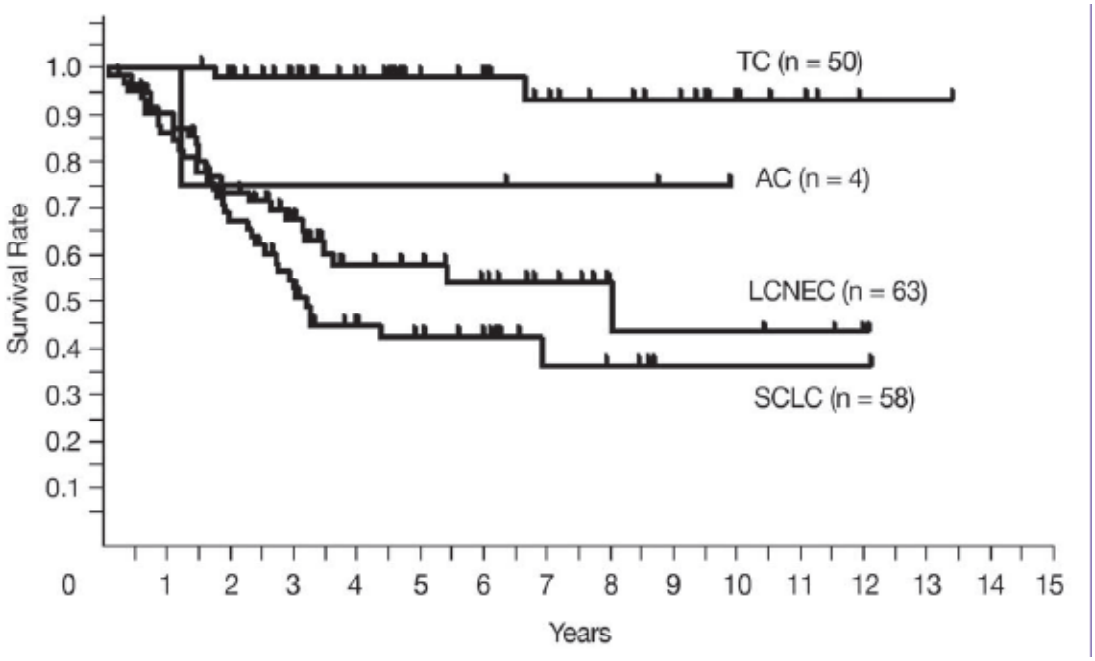
Introduction

Neuroendocrine tumors of the lung include, in the WHO classifications of lung tumors from 1999 and 2004, low -grade tumors represented by Typical Carcinoid (TC), tumors of intermediate grade of malignancy represented by Atypical Carcinoid (AC) and high- grade tumors, which are Large Cell Neuroendocrine Carcinoma (LCNEC) and Small cell Lung Carcinoma (SCLC). They account for 20 to 25% of all lung tumors, the most frequent being SCLC (20%). These tumors harbour histological and immunohistochemical characteristics in common but differ in terms of clinical presentation, epidemiology, genetic, prognosis and survival (figure below). They may be responsible for diagnostic difficulties, carcinoid and particular atypical carcinoid being rare tumors with morphological overlap with LCNEC. The main trouble may also be represented by the quality of the specimens, which can preclude for a definite diagnosis when only cytology or very small biopsies are available.



Overall survival curves in stage I neuroendocrine tumors (n -175) according to the following histologic types: TC, typical carcinoid (n - 50); AC, atypical carcinoid (n - 4); LCNEC, large-cell neuroendocrine carcinoma (n -63); and SCLC, small-cell lung carcinoma (n - 58). The histologic type significantly affected the survival (P- .0001, log-rank test).

From Asamura et al J Clin Oncol 2006.

INVITED LECTURES ABSTRACTS

1. Carcinoid tumors

The mean age for carcinoid tumors is 45-55 years, but they can occur at any age, and with no sex predilection. The most frequent symptoms, observed in less than 50% of the patients, and mainly when tumors are proximal, are dyspnoea, haemoptysis and cough with post obstructive pneumonia. Paraneoplastic syndromes, such as carcinoid syndrome, Cushing's syndrome, and acromegaly can accompany a carcinoid tumor. In 40% of the cases, the tumor is peripheral; when proximal, an endobronchial proliferation is frequently noted by the bronchoscopist. 5% of the patients have an underlying MEN type I disease.

At gross examination, the tumors are very often well circumscribed, with a tan to yellow surface, and measure in average 2 to 3 cm. Under the microscope, carcinoid architecture is typically organoid or with nesting pattern, but other histological patterns include spindle cell features, and trabecular, palisading, glandular, follicular, sclerosing, clear cell, papillary and rosette-like patterns. Cells present uniform cytological features, even if pleomorphism can be observed as well as occasional oncocytic cytoplasms. Stroma can be ossified or calcified.

The criteria for distinguishing AC from TC are the number of mitoses (less than 2 per $2mm^2$ for TC, and between 2 to 10 for AC) and/or the presence of a small punctated necrosis for AC. Diagnosis of carcinoid may be difficult on frozen sections or on small biopsies, where differentiating TC form AC is quite impossible; in addition, carcinoids can mimic metastatic breast cancer or adenocarcinoma., less frequently a sclerosing haemangioma or a melanoma.

Carcinoids express most of the time chromogranin A, synaptophysin and CD 56; they can be cytokeratin negative in 20% of the cases and their positivity with TTF1 antibody is controversial, and perhaps more frequent when carcinoids are peripheral. Usually, Ki 67 staining in TC is observed in less than 5% of the cells, whereas it can be between of 5 to 20% for AC.

The main treatment for carcinoids remains the surgery, with a 5 year survival for TC ranging from 92 to 100%, and from 61 to 88% for AC. Lymph node metastases are found in 4 to 14% of TC and in 35 to 64% of AC at presentation. Of note, TNM staging is now recommended in the seventh UICC/AJCC edition.

As regards molecular and genetic changes, no p53 mutation or Rb loss was found in TC, but they were observed in 25% and 21% of AC, respectively. Both AC and TC have a predominant bax expression. Finally, MEN1 mutations or LOH at 11q13 locus, where maps the MEN1 gene, are found in up to 36% of sporadic carcinoids, mainly AC.

2. Large cell Neuroendocrine Carcinoma

LCNEC are found in nearly 3% of surgically resected lung cancers, and most patients are heavy smokers (> 50 PY). There is also a strong male predominance and the mean age is 62 years. Paraneoplastic syndromes and ectopic hormone production are uncommon. Only 24 % of the patients are asymptomatic and the most common manifestations are chest pain, haemoptysis, cough and fever. Nearly 80% are peripheral. Macroscopically, they are large tumors (one to 12 cm), circumscribed with a necrotic surface.

LCNEC are defined on the basis of histological features suggesting neuroendocrine differentiation, including organoid nesting, trabecular growth, rosettes and perilobular palisading pattern.

Mitotic counts are typically ≥11·2 mm^{-2} (average 75; 10 high-power fields), large geographic areas of necrosis are common, and the cell size (greater than 3 lymphocytes) and the nuclear features differ from those of SCLC. The neuroendocrine differentiation has to be confirmed by the demonstration of positive neuroendocrine immunohistochemical markers (chromogranin, synaptophysin and NCAM CD56), but one positive marker is sufficient if the immunoreactivity is diffuse and clear-cut. In addition, 42% to 75% of LCNEC express thyroid transcription factor TTF-1, which allows assessment of their pulmonary origin; however, expression of cytokeratines 1, 5, 10 and 14 is absent. The proliferation rate is high, with a Ki 67 staining observed in 50 to 100% of the tumor cells.

LCNEC can be combined in 25% of the cases, with associated components of adenocarcinoma, squamous cell carcinoma, giant cell carcinoma and/or spindle cell carcinoma. Combination with SCLC is frequent (~15% of SCLC), and tumors are thus classified as SCLC combined. Of note, making the diagnosis of LCNEC can be particularly difficult on small biopsies or on cytological specimen, and this diagnosis requires a surgical biopsy.

The major differential diagnoses for LCNEC are AT and SCLC; large cell carcinoma, especially with NE differentiation, or basaloid carcinoma have to be considered too. Large cell carcinoma with NE differentiation are defined by large cell carcinoma without NE morphology but positive NE markers; indeed, 10 to 20 % of squamous cell carcinoma, adenocarcinoma or large cell carcinoma can express one positive NE marker (CD 56 more frequently), but often focally. This expression has no influence on the prognosis. Basaloid carcinoma, which can exhibit rosette- like formations, is usually negative for NE markers, but cytokeratin 1, 5, 10 and 14 positive.

The prognosis of LCNEC depends on the presentation stage, which is often III–IV at diagnosis. Clinical prognostic criteria do not differ from other NSCLC, except that tumour spread is relatively more extensive in LCNEC. There is a significantly shorter survival for stage I LCNEC when compared with stage I NSCLC, and stage I large cell carcinoma. Although there has been no significant difference in some series in the prognosis between LCNEC and small cell lung cancer (SCLC) after stratification by stage, the outcome of carefully staged LCNEC may be better than previous studies have indicated.

LCNEC has specific molecular genetics characters similar to SCLC, such as allelic losses at 3p21, fragile histidine triad gene, 3p22.24, 5q21, 9p21, and at Rb gene locus. All these markers correlate with a poor prognosis in these tumours as well as p53 point mutation. LCNEC may carry very similar chromosomal imbalances to SCLC and have P53/Rb mutational patterns that are also shared with SCLC, such as a high frequency of P53 mutation, blc2 overexpression and lack of bax expression, high telomerase activity but lower frequency of Rb, $P14^{ARF}$ loss of protein and E2F1 overexpression. They lack MEN1 mutation and corresponding 11q13 allelic deletion. As SCLC they display a low frequency of P16 loss cyclin D1 and E overexpression. Fas is down-regulated, but its ligand FasL is strongly up-regulated.

3. SCLC

SCLC are the most common pulmonary NE tumors, decreasing in the last 30 years (17 to 13%). Patients are almost all cigarette smokers, and the clinical presentation is characterized by a rapid growth, with mediastinal enlargement and metastases at the time of diagnosis (2/3 of the patients). Rarely SCLC presents as a solitary pulmonary nodule. Symptoms include cough, dyspnoea, haemoptysis, mediastinal compression (vena cava obstruction in 10%) and paraneoplastic syndromes (SIADH syndrome, Cushing's syndrome, autoimmune neuropathy, etc..). TNM staging is also recommended in the 7th edition for SCLC.

The diagnosis of SCLC is mainly made on small biopsies and/ or cytological specimen, because tumors being most of the time unresectable. At histological examination, the tumor consists of dense sheets of small cells (classically not exceeding 3 lymphocytes) with scant cytoplasm, atypical finely granular chromatin with inconspicuous nuclei. Necrosis is common and the mitotic rate is very high, ranging from 60 to 80 per mm^2. Crush artefacts are very frequent but are not pathognomonic. In resected specimen, nearly 30% of the SCLC are combined, with adenocarcinoma, squamous cell carcinoma or LCNEC components. According to the WHO, the diagnosis of SCLC does not require immunohistochemistry and has be made on HE, except when features (histological or clinical) are not classical. The useful immunostainings are cytokeratines and NE markers (CDE56, chromogranin, synaptophysin,), as well as TTF1 (nearly 80% of the SCLC express this antigen). SCLC can also express CD 99, but not CD 45 which can be useful to diagnose a lymphoma. In 10% of the cases, SCLC are negative for NE markers, but if the histological and cytological criteria are consistent with this diagnosis, it has to be maintained.

Prognosis and survival are poor for SCLC, with a 5 year survival around 5%. The treatment remains combination chemotherapy with etoposide with cisplatine or carboplatine.

INVITED LECTURES ABSTRACTS

L18

Anatomy and Development of the meninges: Implications for subdural Collections.

J. MACK

INTRODUCTION:

The dura has traditionally been viewed as a fibrous support membrane containing the dural venous sinuses but otherwise devoid of any specific function. However, inspection of the anatomy of the dura reveals it to be a highly vascular and innervated structure. The rich vascular plexus of the dura likely has a role in CSF homeostasis (1,2). The vessels of the dura are closely associated with sensory nerve endings from the trigeminal. Afferent fibers of the trigeminal in the dura extend to critical nuclei in the brainstem. Activation of these reflex arcs can result in significant cardiac and respiratory compromise with potentially serious consequences (3). Within the collagen fibers of the dura reside multiple fluid channels which have been described as early as 1875 (4,5) but whose function remains unclear. Review of the embryology of the meninges provides a window into the complex developmental anatomy and unique vascularity of these membranes. The intricate structure of the dura implies a purpose that extends beyond that of a fibrous support membrane and emphasizes an important anatomic reality: the dura is not just a cover for the brain but an active participant in its function.

EMBRYOLOGY:

The vasculature of the developing brain and meninges arise within the mesh of neural crest cells surrounding the neural tube. A lattice of primitive capillaries initially envelops the neural tube and from this primordial web emerge the early vessels (6,7). The bridging veins develop early in the first trimester by a process called venous cleavage (7). Initially there are numerous small venous connections between the brain surface and the dural venous drainage. By 6 weeks these primitive vessels have begun to coalesce into larger channels, forming a limited number of large caliber conduits from brain surface to primitive dural sinuses (the primitive bridging veins). The veins intrinsic to the brain and those intrinsic to the dura separate early in development. The early cleavage of these two venous territories is an essential component of the establishment of the blood–brain barrier. The process of venous cleavage reduces the number of pial-to-dural connections, thereby reducing the points at which veins intrinsic to the brain communicate with the systemic circulation.

In contrast to the bridging veins which form early in gestation, the development of the veins of the dura is protracted; definitive arrangement of the dural venous system is not completed until well after birth (6). Perhaps one of the most significant post-natal changes in the dural vascular system is the maturation of the diploic channels which connect the meningeal venous system to the soft tissues of the head. While the diploic venous system is recognizable radiographically at term, it is not fully established until age 5 (8). The superficial, diploic, and meningeal venous vascular territories communicate with each other via the dura mater, and constitute a regulating device for drainage of the cerebral blood (9).

The unique vascular anatomy of the dura provides a distinctive framework within which disease processes that involve the dura can be explored. Two common pathologic alterations of the dura are the accumulation of blood or fluid within its confines (dural hemorrhage and dural effusions).

DURAL HEMORHAGE and DURAL EFFUSIONS:

The dura is contiguous with the arachnoid, and there is normally no space between the layers of the meninges. However, if enough volume of blood or fluid collects within the dura, it can disrupt the inner most layer of the dura (the dural border cell layer) and produce a pathologic collection that is visible on imaging studies.

A subdural hemorrhage is an accumulation of blood within a disrupted dural border cell layer; a subdural effusion is an accumulation of fluid in the same compartment. While bridging vein rupture is traditionally presumed to represent the mechanism by which hemorrhage accumulates in the subdural compartment, it is clear from the anatomy that the intrinsic dural vessels are an alternative source of subdural hemorrhage (10,11). The bridging veins are large caliber and significant force is needed before their tensile strength is exceeded (12,13). They carry large volumes of blood from the cerebral hemispheres, and a traumatic tear of their walls should likewise produce significant volumes of hemorrhage. The vessels making up the intrinsic dural plexus, in contrast, are thin walled, sometimes composed of only a single endothelial cell layer. Bleeding from the dural plexus can expand the dura and disrupt the easily cleaved dural border cell layer (14). The distinction between purely intradural hemorrhage and subdural hemorrhage by imaging can be difficult (14,15). Small volumes of blood within the confines of the dura will change the contour of the thin dural membrane on both CT and MRI. Unless the volume of hemorrhage is significant, it can be difficult to determine whether or not the dural border cell layer has been disrupted.

Causes of hemorrhage from the dural plexus are likely multifactorial. Certainly trauma could result in bleeding and produce SDH. However, SDH is also known to occur without trauma. Bleeding without trauma can occur in patients with coagulopathy of any cause. In addition, genetic syndromes (metabolic disorders as well as those associated with defects in collagen synthesis) can be associated with dural bleeding (16,17). Non traumatic dural bleeding has been described in association with cortical venous and dural venous thrombosis. Vasculitis and vascular malformations that involved the dural vessels are rare, but are another source of dural hemorrhage occurring in the absence of trauma (18-21).

The association of dural bleeding with alterations in CSF volume is not well understood. However, dural collections can occur in states of both increased CSF volume (benign expansion of the subarachnoid space of infancy [22]) and decreased CSF volume (spontaneous intracranial hypotension and overshunting [23]). While subdural collections occurring with these conditions may produce high density hemorrhage on CT studies, more often the collections are hypodense (higher density than CSF, but equal to or lower than the CT density of the brain). Density or signal differences on CT or MRI may simply reflect increased protein content and not blood products. There is likely overlap of these poorly understood collections with the entity known as subdural "effusions."

Subdural effusions are collections of fluid within the confines of the subdural compartment. One of the most well studied causes of dural effusions are those that occur in association with meningitis. Effusions occurring in the context of meningitis are common, with incidence estimates as high as 50% (24). The fluid is more proteinaceous than CSF and can be hemorrhagic or xanthochromic. Density and signal intensity on imaging can vary. The accumulation of fluid is thought to be the result of altered CSF dynamics within the dura (24).

Only rarely are cultures of these effusions positive, even when there is extensive infectious involvement of the arachnoid. The mechanisms by which the dura produces an effusion is of some physiologic interest, and may point to a possible role of the dura in immune function. For example, it is well known from the migraine literature that neuropeptides released from intradural trigeminal nerve endings produce plasma extravasation from the dural venules. The plasma extravasation is exacerbated by degranulation of resident dural mast cells (25). If the plasma extravasation is accompanied by alteration in the normal fluid and CSF dynamics of the dura, the volume of fluid may be great enough to accumulate in the subdural compartment. It is clear that subdural effusions seen with meningitis are a response to the underlying inflammation of the arachnoid membranes is; the origin of that response may be neurogenic inflammation mediated by the trigeminal nerves, resident mast cells, and the dural venules. Finally, while an effusion may be easily seen on imaging, and aspirated using a subdural tap, it may be missed on pathologic evaluation, immediately dissipating when the skull and dura are reflected from the arachnoid during post-mortem examination.

It is important to incorporate both dural effusions and dural hemorrhage in any discussion of subdural collections seen on imaging, though doing so necessarily complicates the differential diagnosis of imaging detected "SDH." Low-density dural collections are frequently referred to as "chronic" subdural hemorrhages when seen on CT exam, implying that there was hemorrhage at some remote time in the past. However, not all of these low-density collections are associated with prior hemorrhage (26). While multiple layers of dural fluid of different density or signal intensity can be seen in patients with chronic collections and re-bleeding, the radiographic finding of mixed density collections is by no means specific for chronicity (27). Mixed density can be seen in acute dural hemorrhage, and non-hemorrhagic dural effusions can be seen in acute traumatic and non traumatic settings (28,29). To complicate matters further, non-hemorrhagic effusions can progress to become chronic collections (30,31).

CSF CIRCULATION:

A discussion of the dura is not complete without reference to CSF circulation. The adult brain manufactures approximately 500 cc's of CSF per day, the majority of which is secreted by the choroid plexus into the ventricular system. The CSF communicates with the interstitial compartment of the brain parenchyma, and circulates through the ventricles and subarachnoid space. Normal CSF circulation is essential for normal function of the brain. The primary location of absorption of CSF is a matter of significant debate. While the traditional view holds that arachnoid granulations provide the main route of transport from the CSF compartment into the systemic circulation, it is clear that other routes of absorption also play a significant role, particularly in the infant (32). The final route of exit in all instances necessarily involves some kind of pathway through the dura. While the dura has traditionally been considered devoid of lymphatics, recent work with lymphatic immunohistochemical markers has suggested lymphatic like vessels in the dura of the human optic nerve (33). In addition, recent work by Squier has revealed a complex and developmentally dependent network of dural channels (34). Also described in the historic dural literature, these channels are thought to participate in fluid movement through the dura. This possible function of the dura as a "fluid transporter" is supported by staining of the dura with the water channel marker, aquaporin 1 (35 and Squier). Understanding the dura's role in fluid and/or CSF

transport will lead to better understanding of the mechanisms by which dural collections occur in states of altered CSF volumes.

CONCLUSION:

The dura is more than a fibrous covering to the brain. Its intricate anatomy includes interwoven and communicating vascular territories that drain the brain, supply the skull, and communicate with the superficial soft tissues of the head, face and neck. It is supplied by a complex network of sensory nerves that respond to pressure, temperature, and changes in pH balance. It is home to dural macrophages and mast cells and may play a significant immunologic role in the CNS. Within its fibrous layers is a complex lymphatic like collection of "channels" that likely participate in fluid and CSF homeostasis. Disruption of any of the components of the dura has the potential to result in pathologic alterations of its structure. Such alterations include bleeding from the dural vessels or fluid extravasation from the fluid channels or from the dural venules. If the volume of such bleeding or fluid extravasation is great enough, it will be picked up on imaging studies as an abnormal appearance of the normally thin contour of the dura. Only through careful radiologic, clinical, laboratory, and pathologic correlation will we begin to unravel the causes of such anatomic disturbances.

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INVITED LECTURES ABSTRACTS

L19

Diagnostic electron microscopy: past and future

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Introduction

Looking back at the history of diagnostic electron microscopy (EM), a dramatic change of paradigms can be seen. Roughly, four phases can be discriminated. During the early phase (1940-1960), EM viral diagnosis was confined mainly to differentiating smallpox from other viruses present in vesicle fluids of skin lesions. During a second phase (1960-1980), so far the broadest application of EM in viral diagnosis, many new viruses were described and taxonomy of viruses was developed based on morphological criteria. The third phase (1980-2000) is characterized by a marked, worldwide reduction of laboratories performing diagnostic EM. Following the rapid development of modern test systems based on both molecular biology and genetics, the use of diagnostic EM was considered to be redundant in an increasing number of institutes; the more because of its inherent quantitative and economical limitations EM is not suited for mass screening of clinical specimens. In addition, the first and second generations of electron microscopists were often not replaced when they retired, and funds for equipment were directed towards the acquisition of confocal microscopes (which lack resolution for viral diagnosis), while older electron microscopes were not replaced.

During the last 10 years, outbreaks of several viral zoonoses with an often fatal result for man have strongly determined the actuality. These include Hendra and Nipahparamyxoviruses, SARS coronavirus, monkeypoxvirus, Ebola and Marburg virus, avian influenza and West Nile flavivirus. In all of these cases, the EM-diagnostic was decisive or important, either as a first-line test, or to give an independent confirmation of the results obtained in other tests based on virus morphology. Application of EM during the next outbreak of an emerging virus is almost sure. In this context EM-units are an important asset for orientating and confirmatory diagnosis of infectious diseases, provided that their organization is adequate and that specific expertise is developed. In recent years, the need to characterize recombinant viruses and virus-like particles is strongly augmented driven by their increased application in vaccines. Electron microscopy, supported by advanced software (semi-automatic particle analysis and electron tomography) or by antigen-specific labeling (immunogold staining) provides the required resolution to allow analysis on a per-particle basis.

Methodology

Successful EM diagnostic requires continuous training of pattern recognition capabilities. This is the ability to carry mental images and compare them with what is visible on the screen. It also requires the ability to see whether the observed image is compatible with what is known of a particular virus and whether it is incompatible. It is necessary to maintain this capability by examining a variety of pathogens, including pathogens in field samples, on a regular basis. In laboratories, where samples are diagnosed only occasionally and daily routine is lacking, it is very difficult to keep the quality of EM diagnoses high, resulting in further reductions of this activity.

INVITED LECTURES ABSTRACTS

To exploit the potential of diagnostic electron microscopy fully, state-of-the-art methodology should be used, its quality controlled and it should be applied as a frontline method coordinated and run in parallel with other diagnostic techniques.

Negative staining and ultra-thin sectioning are the standard methods in a diagnostic EM setting. Negative staining is a technically simple and rapid procedure for EM-based on staining with heavy metals. This technique allows visualizing bacteria, macromolecules, surface components of cells, internal cellular organelles, and viruses by transmission electron microscopy. The technique comprises of coating particles in suspension to a film-coated grid, rinsing excess material from the grid, staining the grid with a heavy metal-based stain, followed by electron microscopic examination. Negative staining and the evaluation at the electron microscope need less than 30 minutes until a diagnosis is met – provided the necessary high particle concentrations (10^7 particles per ml) are present in the suspension and that the samples do not require an additional fixation or enrichment. The technique of ultra-thin sectioning for examining biological materials allows to embed the material under study in plastic and cut ultra-thin sections that can be examined by transmission electron microscopy (TEM). Probably the most critical step in the whole process is the fixation. By fixation a rapid cessation of biological activity and a preservation of the structure is brought about. Ideally, the colloidal suspension of cytoplasm and organelles within a cell are turned into a gel that maintains the spatial relationship of the components while providing sufficient stability for them to survive the solvent action of aqueous buffers, dehydration agents, and plastic resins. After fixation, the material to be sectioned is contrasted with solutions of heavy metal salts (osmium tetroxide and uranyl acetate), dehydrated in ethanol or acetone, and embedded in plastic (epoxy resin). Ultrathin sections (60 nm) cut with glass or diamond knives using an ultramicrotome are floated on water, transferred to specimen support grids and examined in the TEM. Often the sections are further contrasted with uranyl acetate and lead citrate prior to examination in the microscope.

Results and discussion: diagnostic EM in practice

For routine detection of specific viruses, alternative tests like ELISA and PCR techniques should be preferred above diagnostic EM. To clarify "difficult" syndromes and as an instrument of rapid viral diagnosis, EM is however hardly to be substituted, particularly if the gain in time is an important factor in the control of infectious diseases. In the laboratory diagnosis of complex, rare or new infectious diseases, diagnostic EM shows three principal advantages: it has an undirected "open" view, it can excel by its rapidity, and it provides information that is independent from that obtained by most other tests, because it is based on morphology. Regrettably, diagnostic electron microscopy is often considered only as a final resort. Often the failure of a series of specific diagnostic tests is required before a veterinarian or a laboratory worker decides to search for one of the few remaining EM units specialized in the diagnosis of infectious agents. The remaining sample might, but often is not, be taken and fixated properly, but expectations invariably are that, as a *deus ex machina*, this beautiful piece of machinery produces a picture of text book quality showing the long-searched-for etiological agent. Remarkably, and thanks to some weird human intervention, such whishes are not seldom granted and the scientist returns to his lab happily in the belief that in case of emergency, he always can turn to the electron microscope as a final resort.

Indeed, in this era of avian influenza, a simple negative staining allowed to visualize rota-, adeno-, pox-, reo-, paramyxo-, gumbooroo-, and herpesviruses in cultures or embryos

that were clearly virus-infected, but contained no influenzavirus. Such results were not only obtained in chickens, but also in exotic species like partridges, pigeons and ducks, where virus-specific tests are often unavailable. Also, in addition to the usual suspects, like mycoplasms and endogenous retroviruses, contaminant bovine viral diarrhea virus and simian paramyxoviruses are found in stock solutions of other viruses and even in uninfected cell lines. If no 'open-view' method, like diagnostic EM, is used, these co-infections can persist unnoticed for months, influencing virus titration and seroneutralisation tests.

In certain cases, no etiological agent can be demonstrated, but interpretation of a changed cellular ultrastructure allows reorientation of the investigations.

In the autumn of 2003, more than 40 horses, all free ranging on meadows in the North of France and the South of Belgium, suddenly died due to an atypical myopathy.

Compared with control animals, affected horses demonstrated an accumulation of large intracellular lipid droplets and an open structure of the mitochondria, indicative for mycotoxin intoxication. This hypothesis agrees nicely with the humid climatological conditions, with the observation that only free-ranging animals were affected, and with the observation that ruminants sharing these pastures were not affected. Ruminants are less sensitive to such intoxication due to destruction of mycotoxins by the microflora of the rumen. Similarly, an increased mortality of broiler breeder chickens, was recently observed in several farms in Flanders (Belgium). EM diagnosis demonstrated no infectious agent, but ultra-thin samples showed a disturbed fat metabolism of hepatocytes. Symptoms of fatty degeneration of hepatocytes varied from advanced cell swelling, accumulation of large triglyceride globules and complete ultrastructural decay of metabolically active parenchymal hepatocytes while structural fibroblasts and erythrocytes remained intact. As a result, factors influencing fat metabolism are examined instead of chasing an illusive infectious agent. These include severe food restriction, imbalances of feed nutrients and co-enzymes, contaminating mycotoxins and chronic hypoxia due to selection for high metabolic activity.

All above-mentioned cases arrived in the EM-lab with a history of negative results in specific tests, before EM was considered, and found useful, to obtain an orientating diagnosis. Ironically, an identical result would have been obtained, but much faster and more efficiently, if diagnostic EM would have been applied as a frontline test, rather than as a final resort.

In specific cases, results of conventional EM preparation techniques can efficiently be complemented by the results of advanced EM-techniques. The combination of negative staining of complex viruses with electron tomographic analysis, allows visualizing and measuring artifacts typical for negative staining. This approach further allows sharp visualization of structures in a subnanometer-thick plane, avoiding blurring due to superposition, which is inherent to TEM. The combination of negative staining with immunogold labeling using specific antibodies allows distinguishing mutant paramyxoviruses and recombinant feline herpesviruses from the corresponding parental strains. Finally, the combination of negative staining with semi-automatic detection and analysis of particles allows characterizing virus-like particles quantitatively.

INVITED LECTURES ABSTRACTS

L20

New insights in gestational trophoblastic disease (GTD)

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It is not unfair to say that trophoblastic lesions continue to pose diagnostic problems for pathologists. It is not the intention of this communication to provide a complete overview of all forms of GTD included in the latest WHO classification. I will rather focus on recent developments and new conceptual insights in GTD, that could be of interest for the practising histopathologist.

The most common diagnostic error in GTD is the underdiagnosis of an early complete mole as a partial mole. The classic features of "textbook" complete moles are 1) markedly enlarged, balloon-like villi, 2) villous cavitation with cistern formation, 3) marked cyto- and syncytiotrophoblastic hyperplasia, and 4) trophoblastic atypia. However, these complete moles were only diagnosed at a mean gestational age of 17 weeks. Nowadays, with the use of improved ultrasound equipment, pregnancies suspected for mole are evacuated at 8 to 10 weeks. In early gestations, the classic features of complete mole simply do not have the time to develop. A new set of histologic criteria for early complete mole is therefore required: 1) a myxoid (mucoid) and hypercellular stroma, resulting in a polypoid almost phylloides-like villous configuration, 2) villous stromal karyorrhexis, and 3) loss of $p57^{kip2}$ expression. Villous stromal karyorrhexis is highly characteristic. It is the consequence of increased proliferation and subsequent apoptosis of stromal cells.

The use of immunohistochemical staining for $p57^{kip2}$ protein has revolutionized our ability to diagnose early complete hydatidiform mole. This protein functions as a cell cycle inhibitor and a tumor suppressor. The gene is paternally imprinted, so that expression from the paternal allele is suppressed. Complete moles are almost always diploid*, with a complete paternal genome; they are "uniparental" and "androgenetic". Consequently, in a complete mole, p57 is negative in the majority of villous cytotrophoblastic and stromal cells. Partial moles (and non-molar conceptions) are biparental; because the maternal allele is expressed, they show strong nuclear p57 staining in the majority of villous cytotrophoblastic and stromal cells. A possible source of confusion is the fact that even in a complete mole, there is strong nuclear staining of extravillous trophoblast. There is no satisfactory explanation for this phenomenon.

*A small subset of complete moles is biparental. The characteristic history is recurrent familial complete hydatidiform mole, and recently the gene has been localized on 19q13.4. The disease has an autosomal recessive transmission pattern. p57 is negative in these cases.

Obviously, immunohistochemistry is not helpful in the differential diagnosis between partial mole and hydropic abortion. According to Chew et al., a partial mole can reliably be diagnosed when at least 3 of the following features are present:

Partial mole or aneuploid gestation:

- dual population of admixed enlarged villi with small normal-appearing villi
- trophoblastic inclusions
- scalloped villous borders
- trophoblastic hyperplasia, largely restricted to syncytiotrophoblast
- cisterns

Chew et al., *Hum Pathol* 31: 914-924, 2000

Aneuploid gestations will display only 1 or 2 of these features. A hydropic abortion *not otherwise specified* shows variably sized immature villi with a smooth contour and attenuated trophoblast, polarized trophoblastic proliferation without atypia, and no central cistern formation.

Persistent trophoblastic disease (PTD) is not an easy concept. It is a clinical diagnosis, not a histopathological one. In fact, the diagnosis of PTD is often made without tissue diagnosis. If nonetheless a curettage is performed in the follow-up of a molar pregnancy (because of HCG plateau or rise) it may show persistent hydatidiform mole, choriocarcinoma, retained implantation site**, no evidence of trophoblastic tissue, or rarely a new pregnancy. In the majority of cases, PTD represents an invasive mole. Other possibilities include choriocarcinoma, placental site trophoblastic tumour (PSTT) or epithelioid trophoblastic tumour (ETT). PTD is also called "malignant trophoblastic disease", despite the fact that most cases are not choriocarcinoma. Only 10 to 20% of patients with postmolar GTD have choriocarcinoma. Clinically PTD is further classified in nonmetastatic and metastatic (low risk, and high risk). This classification determines prognosis and treatment.

**Another common diagnostic error in GTD is the overdiagnosis of placental implantation site as placental site trophoblastic tumour or choriocarcinoma. Placental site intermediate trophoblast can be quite exuberant ("exaggerated") or atypical, especially in the context of a complete mole. Such atypia is of no clinicopathological consequence.

Choriocarcinoma, PSTT and ETT are grouped by the FIGO as (malignant) gestational trophoblastic neoplasia. It becomes increasingly evident that malignant trophoblastic neoplasia is less homogeneous than previously thought. Hybrid or mixed variants with combined features of both choriocarcinoma and tumours of intermediate trophoblast (IT) are not infrequent. After neoplastic transformation of trophoblastic stem cells (presumably cytotrophoblast), specific differentiation programmes dictate the type of trophoblastic lesion that develops. Choriocarcinoma is the most primitive trophoblastic tumour, whereas PSTT and ETT are more differentiated.

Immunohistochemistry should be used with caution, bearing in mind that it is never a substitute for careful clinicopathological correlation, and that too much faith in differential staining can be misleading in small tissue samples. The recent literature emphasizes (perhaps

too much) the role of immunohistochemistry in the differential diagnosis of lesions and tumours of IT. I favour a pragmatic approach in 4 steps: 1) Confirm the trophoblastic nature of the lesion. CK18 is intensely positive in trophoblast, while completely negative in decidua. Inhibin- is expressed by all populations of trophoblast, except cytotrophoblast, and in all trophoblastic lesions. 2) Eliminate choriocarcinoma as a diagnostic possibility. It shows the characteristic bimorphic appearance with malignant cyto- and syncytiotrophoblast, the latter staining intensely positive with -HCG. 3) Distinguish between implantation site IT (HPL+++, p63-) and chorionic type IT (p63+++, HPL -/+).

However, the distinction between PSTT and ETT is not important clinically. 4) Determine benign versus malignant. When using Ki-67, double staining with Mel-CAM is imperative to positively identify the Ki-67 positive cells as IT, since many Ki-67 positive cells in the implantation site ar not trophoblast (T-cells, endometrial granulated lymphocytes).

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INVITED LECTURES ABSTRACTS

L21

The borders between Hodgkin and non-Hodgkin lymphomas.

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In the last years, it has become evident that although most lymphomas can be classified as distinctive disease entities, there are some cases, which show overlapping morphological, biological and clinical features between various types of lymphomas. The 2008 World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues (1) recognized these problematic and introduced two provisional categories of B-cell lymphoma, unclassifiable, one with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) (Kluin P et al, WHO), and the second one with features intermediate between DLBCL and classical Hodgkin lymphoma (CHL) (Jaffe ES et al, WHO).

This lecture will cover exclusively the second provisional category.

The second provisional category relates mainly to the distinction of mediastinal CHL, nodular sclerosis subtype with DLBCL, usually primary mediastinal large B-cell lymphoma (PMBL). Although in most cases one or the other diagnosis can be made, there seems to be a true biological "grey zone" between these two entities, as shown by gene expression profiling where both entities revealed striking similarities (2-3). Although this category includes mainly lymphomas in young patients with mediastinal disease, similar cases have been reported in peripheral lymph nodes, as a primary site in adult population. Current knowledge accepts that CHL is derived from an altered B-lymphocyte characterized by an abnormal B-cell program, and incapability to secrete immunoglobulin. Therefore, it is not surprising that biological or clinical overlap should occur between CHL and B-cell NHL. The first cases reported in the literature dealt mainly with two scenarios. One group included cases with morphology resembling CHL but have atypical features such as increased numbers of large mononuclear cells, lack of the typical mixed inflammatory background, and atypical immunophenotypic characteristics of the Hodgkin-like cells such as strong expression of CD20 and expression of the transcription factors OCT-2 and/or BOB.1. The second group was characterized by cases with typical morphology of PMBL including diffuse monomorphous large cells, compartmentalizing sclerosis and sparse inflammatory background. However, the immunophenotype in these cases was atypical and suggestive of CHL with weak or lack of CD20 expression, very often the neoplastic cells were positive for CD15 and the B-cell transcription factors (PAX5, OCT-2, BOB.1) were usually expressed.

The 2008 WHO classification created a new category to accommodate these cases in which the distinction between CHL and DLBCL was not possible. The majority of these patients are young males with mediastinal masses, in contrast to both NSCHL and PMLBCL, which are more common in females. Most cases are characterized by the expression of CD45, a preserved B-cell program (OCT-2+, Bob1+, PAX5 strongly expressed, (unlike the weak positivity found in CHL), with strong expression of CD20 and/or CD79a, and aberrant expression of CD30 and CD15 in the majority of the cases. The optimal therapy for these patients has not been determined.

INVITED LECTURES ABSTRACTS

In order to review controversial issues in "grey-zone" lymphomas, a joint Workshop of the European Association for Haematopathology (EAHP) and the Society for Hematopathology (SH) was held in Bordeaux, France in September 2008. The conclusions and recommendations of this meeting were recently published (4). First, it was concluded that in order to achieve an accurate diagnosis, all information, including cytomorphology, biology and clinical data should be considered, and second, whenever possible, distinct entities should be diagnosed. It was agreed; however, that this was not possible in all cases, thus justifying the diagnoses of a borderline lymphoma (unclassifiable) with features intermediate between CHL and DLBCL. It was stressed that deviation of a single marker or criterion, e.g. strong and uniform CD20 expression in otherwise typical HL, was not sufficient for placing a case in the borderline category, but that a hybrid phenotype not being in line with current morphologic and/or biologic concepts would well be. Examples of those features could be a significant mononuclear large B-cell component not compatible with HL, or uniformly strong expression of pan B-cell markers along with the expression of one or more of the transcription factors BOB.1, OCT-2 and PU.1 in a mediastinal tumor with morphological features of CHL. This stresses the need of doing a complete phenotype (several B-cell markers and transcription markers) in cases with morphological features of CHL with strong and uniform CD20 expression.

A group regarded as "grey zone" lymphoma, although not included as a provisional category in the 2008 WHO classification, deals with the relationship between nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and T-cell histiocyte-rich large B-cell lymphoma (THRLBCL). Because NLPHL and THRLBCL derive from follicular center B-cells, problems in the differential diagnosis may occur (5). The immunophenotype of the tumor cells, do not differ significantly, however, the small reactive B-cell background and the growth pattern (nodular Vs diffuse) is of great help for the differential diagnosis, although borderline cases with overlapping morphology may occur.

Similar to the mediastinal "grey-zone" group, cases with CHL and DLBCL borderline features, mainly THRLBCL, also exist in lymph nodes. Accurately separating cases of CHL from THRLBCL due to overlapping clinical, morphological and/or immunophenotypic features might be difficult. Additionally, the frequent association of these cases with EBV also discloses overlapping features with (THRBCL-like) EBV-associated DLBCL of the elderly. Similar problematic can be found in young patients.

A similar but unrelated diagnostic issue involves those cases in which there may be diagnostic uncertainty, but not true biological overlap. The interface between some peripheral T-cell lymphomas (PTL) and CHL may be ambiguous histologically and immunophenotypically, but does not represent a biological "grey zone". (6) Angioimmunoblastic T-cell lymphoma and Anaplastic large cell lymphoma are the two T-cell lymphomas, which most commonly show Reed-Sternberg-like cells.

The lecture will discuss some of the most problematic borderlands from clinical and diagnostic standpoint.

- Classical Hodgkin lymphoma and primary mediastinal large B-cell lymphoma
- NLPHL and T-cell/histiocyte-rich large B-cell lymphoma
- T-cell/histiocyte-rich large B-cell lymphoma and classical Hodgkin lymphoma

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INVITED LECTURES ABSTRACTS

L22

Tick-borne encephalitis in Europe: Review of an emerging zoonosis

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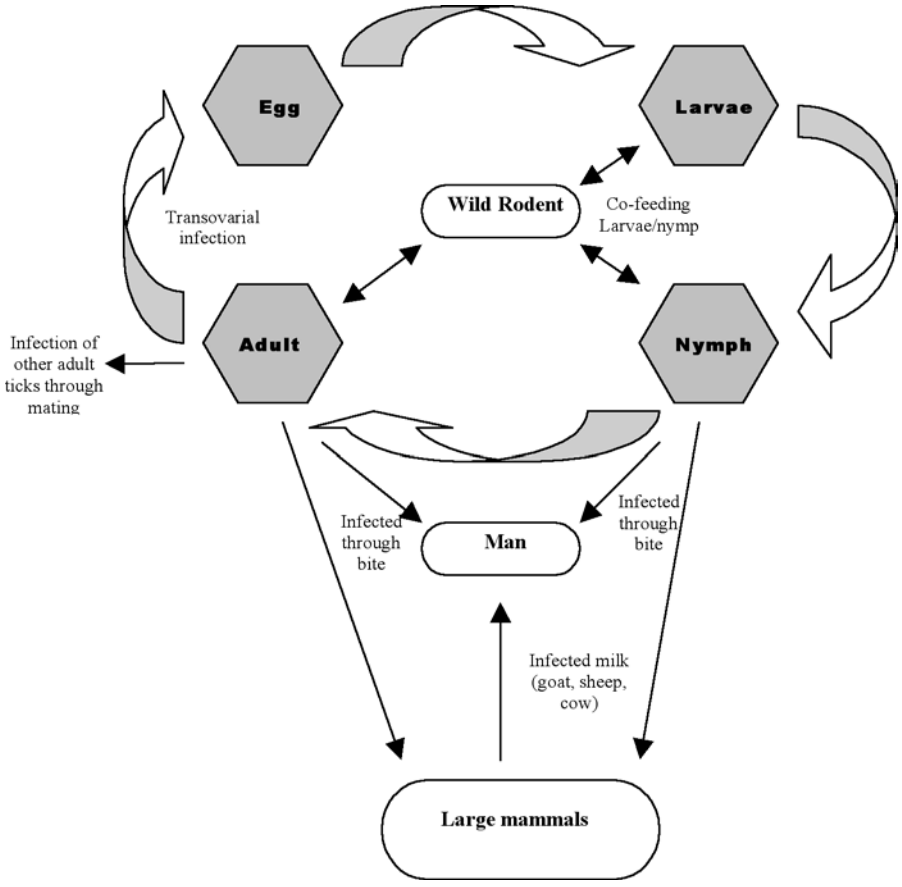
Tick-borne encephalitis virus (TBEV) is the most important arthropod-borne virus in Europe. In Europe, the Western subtype of this highly pathogenic neurotropic flavivirus is carried by *Ixodes ricinus*. Tick-borne encephalitis (TBE) has become a considerable public health risk in several European countries, with currently 3000 hospitalized cases per year. In many patients the disease results in long term sequelae and disability. Recent increases and fluctuations in human incidence in Central and Eastern European countries (e.g. Switzerland, Germany, Poland, Baltic States) and the emerging of the disease in Finland, Norway, Denmark and France have sparked international concern and research. TBE is also emerging in Europe's canine population and numbers of clinical cases in dogs are expected to increase;

TBEV is a small spherical enveloped RNA-virus that belongs to the genus *Flavivirus* (*Flaviviridae*), which contains many neurotropic pathogenic arthropod-borne viruses. Its genome consists of a 10.5kb (approximately) single positive strand of RNA, encoding three structural and seven non-structural proteins. Three lineages of TBEV have been described, namely the Western subtype transmitted by *I. ricinus* ticks and the Siberian and Far Eastern subtypes transmitted by *I. persulcatus*. Besides the co-circulation of all subtypes in Estonia and Latvia and one focus of the Siberian subtype discovered in Finland, only the Western subtype is present in Europe.

The clinical manifestations in human cases have been well-documented and there is a range of symptoms that can be observed. Although the far-eastern subtype of TBEV causes a monophasic course of illness, infection with a western European subtype usually produces a biphasic course of illness. The incubation period is generally 7 – 14 days and during a typical biphasic infection, symptoms during the initial short febrile period can include fatigue, headache and pain in the neck, shoulders and lower back, accompanied by high fever and vomiting. This is often followed by an asymptomatic period lasting 2 to 10 days and if the disease progresses to neurological involvement, this leads to the second phase. CNS infection can manifest in the meninges (meningitis), the brain parenchyma (encephalitis), the spinal cord (myelitis), the nerve roots (radiculitis) or indeed any combination of these. Acute TBE is characterized by encephalitic symptoms in 45 to 56 % of patients.

Symptoms range from mild meningitis to severe meningoencephalomyelitis, which is characterized by muscular weakness (paresis) which develops 5 to 10 days after remission of the fever. Severely affected patients demonstrate altered consciousness and a poliomyelitis-like syndrome that may lead to log-term disability.

Human TBE is emerging in several Northern and Western European countries and several canine TBE cases were diagnosed in dogs living in or visiting endemic areas. Given the dramatic increase in human cases in recent years, TBE is likely to be more frequently diagnosed in dogs in the future as awareness in the veterinary community rises. Though Belgian dogs travel to endemic areas and though dogs tend to have more frequent contact with the tick vectors of TBE (*Ixodes ricinus*), clinicians do not routinely test for TBE in canine meningoencephalitis cases and veterinary surveillance is currently non-existent in Belgium.



Transmission of tick-borne encephalitis virus within the life cycle of Ixodes ricinus

(tick stages are highlighted with grey shading)

(Mansfield et al, 2009)

Until now, no autochthonous human or canine cases were reported in Belgium, but if TBE were to emerge, it could pose an important threat to canine and public health. Targeted serological screening of sentinel animals such as dogs and wildlife would contribute in a cost-effective way to a continuous epidemiosurveillance program for TBEV in Belgium.

Serum samples of Belgian dogs (n=713) were obtained from three diagnostic laboratories from northern (n=521) and southern (n=192) Belgium (n=713) in different provinces (West Flanders, n=401 ; East Flanders, n=120 ; Liège, n=192). All samples were drawn by local veterinary surgeons between 15/03/2009 and 31/05/2009 and submitted to the laboratories for a variety of diagnostic tests. Samples were centrifuged and the sera were stored at 4°C at the laboratories until collected, at which time they were frozen and stored at -20°C. All sera were screened for anti-TBEV-IgG with the described ELISA protocol following the manufacturer's instructions. Positive, borderline and near-borderline samples were subjected to a rapid fluorescent focus inhibition confirmation test. One dog was found positive. The other ELISA-positive samples underwent neutralization tests to rule out West Nile and Louping Ill viruses and were found to be negative. A retrospective descriptive analysis of the history of the seropositive dog did not conclude beyond doubt whether TBEV infection was acquired abroad or in Belgium, hence both scenarios remain possible. Further surveillance and investigation is necessary to determine whether this dog remains a single "travel-related case" or whether she is "the tip of the iceberg".

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INVITED LECTURES ABSTRACTS

L23

High grade intraepithelial squamous lesions

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Cervical cancer represents the second most common cancer among women and a leading cause of mortality worldwide, with 273.000 deaths estimated in 2002 (1). Eighty-three percent of cases occur in the developing world, where cervical cancer accounts for 15% of female cancers, as compared to just 3.6% in developed countries (1).

The large decline in cervical cancer incidence and mortality in high-income countries is largely credited to effective screening programs (2-3).

The first organised screening programmes in Europe were started in 1959 (Norway) and 1960 (Scotland). Since then cervical cancer screening programmes have been introduced in almost all the member states of the European Union (opportunistic, pilot, organised etc ..) (4).

Cervical cancer screening started in Italy as a spontaneous activity, in the absence of organised programmes; this led to incomplete population's coverage. In 1996 the National Oncologic Commission delivered national proposals and guidelines for cervical screening as part of the National Health Plan (5). Organised Screening Programmes on a regional basis were advocated, recommendations based on EC " Guidelines on quality assurance in cervical cancer screening" (6) included: personal invitations, quality assurance for each phase of the programme and the presence of an evaluation system. In September 1996 an Italian Group for Cervical Cancer Screening was founded to try to join existing organised screening programmes. Following national guidelines organised regional screening programmes have started throughout the country at different times and with different organisations according to the different realities: centralized regional programmes, provinces based programmes, local health units based programmes. Nowdays, only one region is still working on starting an active programme.

An organised screening programme has been active in our region (Friuli Venezia Giulia) since 1999, women aged 25-64 are invited to take a Pap test every three years; the third screening round has been completed in 2007. Target population has been always stable, with an average of 330.000 women. Compliance to screening invitation has been increasing; with an average of 56.3% in the third round. Improvements in coverage percentage have also been observed, 62.3% was the value in the third screening round; also if there is still a part of women who has not been given a Pap smear or has been given it in a private structure.

In our region, where we have six public laboratories, analyzing Pap smears coming from 42 sampling centers, a computerized reporting system is used. This allows continuous monitoring of the main quality indicators by the Screening Regional Agency, which can work out general quality of screening programme.

As far as reporting, Bethesda System 1988-91 has been adopted until 2007, we have started adopting Bethesda System 2001 in 2008. We have introduced HPV test for ASC-US cases and for post conization follow up since 2009 as HPV tests in combination with Pap tests are 96%

to 100% sensitive for detection of cervical intraepithelial neoplasia (CIN) 2/3 and cancer and it represents an alternative to colposcopy and/or cytology for follow-up of treated cases. Proper use of HPV testing improves the management of women with cytologic abnormalities. (7)

Pap test's main aim is to detect high grade squamous intraepithelial lesions which morphologically show a variety of patterns that may be source of diagnostic controversy.

They are characterized by a population of moderately sized parabasal (CIN 2) or basal (CIN3) dysplastic cells with marked nuclear abnormalities occuring singly or in clusters; not all dyskaryotic cells have hyperchromatic nuclei (pale dyskariosys) and their identification can become challenging. As the parabasal dysplastic cells may resemble benign metaplastic squamous cells, they have also been described as " atypical immature squamous metaplastic cells" (8), but this term may be higly misleading and it may create confusions with clinicians. Basal dysplastic cells usually display scanty, barely visible, basophilic cytoplasm and large hyperchromatic, coarsely granular nuclei with irregular contours. These elements can be detected singly or in clusters also depending on the technique of cytologic sampling, and if the centers of clusters are way too dense for analysis, the study of their periphery will help to disclose the nuclear abnomalities of dysplastic cells. It has to be stressed that basal dysplastic cells represents one of the most difficult lesion to be identified; they can be mistaken for lymphocytes (follicular cervicitis), macrophages (because of the similar arrangements in strings or files) or for small benign metaplastic or endometrial cells (due to their similar sizes). Regressive changes in endocervical cells can be confused with HGSIL. Repair cells can be source of confusion, but they are characterized by active nuclei with nucleoli and even distributed chromatin. High grade dysplastic lesions involving glandular structures may exfoliate in sheets and aggregates arising diagnostic doubts with glandular lesions. High grade keratinizing squamous lesions are characterized by keratin-forming dysplastic cells of different shapes with abundant orange or yellow thick cytoplasm. "Tadpole" (caudate) cells, spindly shaped cells and squamous "pearls" can be also observed. Nuclei are often picnotic or sometimes they can be completely or partially replaced by keratin ("ghost" cells). Excessive anucleated keratin material coming from the superficial part of lesions could hide either high grade lesions or benign leukoplakia. In these cases, an adequate colposcopic evaluation with biopsies become extremely important for this group of lesions. Keratinizing high grade lesions could also be hard to differentiate from invasive cervical cancer; detection of necrotic background in invasive lesions is helpful for diagnosis. On the other side, clean background is more suggestive for intraepithelial lesions.

High grade squamous intraepithelial lesions may also have some special morphologic presentations in particular conditions such as postmenopausal atrophy. Interpretation of atrophic smears is often a source of diagnostic challenges because atrophy and dryness have several effects on dysplastic cells. They appear enlarged, their nuclei appear pale and the chromatine texture is not well discernible; diagnosis can be performed by careful comparison of abnormal cells with adjacent dry, but normal cells. On the other side a markedly atrophic benign smear with enlarged or elongated spindly squamous cells (as it may happen in case of bad spreading on smears) may suggest the presence of a dysplastic lesion even to experienced observers. If nuclear sizes and texture of suspect cells are closely similar to those of benign cells, high grade sil lesion diagnosis is not suggested. If doubts persist, the simplest solution will be to refer patient for colposcopy with ASC diagnosis.

Pregnancy represents another important challenge in detection of squamous intraepithelial lesions; abnormalities caused by florid squamous metaplasia can be easily mistaken for dysplastic lesions and extensive cytolysis may destroy the cytoplasm of abnormal cells (9).

Also if Pap test is not perfect and false-negative results cannot be completely eliminated because of various factors which limit test sensitivity, we would like to stress the importance of cytology coming from our experience.

In our laboratory, in Trieste, both cytological and histological gynaecologic specimens from the entire province are processed, this allows daily cyto - histological comparison, which represents an essential element for cultural growth, expecially for discrepant cases. In some cases of positive cytology for High Grade Squamous Intraepithelial Lesion with following negative biopsy, Pap smear is reviewed, and if HG lesion is confirmed, after communication with clinicians, patient is referred for large loop excision of the transformation zone (LLETZ) or conization which usually confirms presence of high grade lesion detected by cytology, but missed by colposcopy mainly because of their upper endocervical or endoglandular localization.

Good results obtained by cervical screening in our area are also demonstrated by one of our studies starting from 102 invasive cervical cancers observed in Trieste area from 1999 to 2008. Most (80%) of the patients had never been given a Pap smear, 17% had not followed Italian Cervical Screening guidelines, the remaining 3% had been diagnosed a HG lesion through cytology, but they did not respond to second level exams (colposcopy, biopsy and therapy).

This is to highlight one of the most important risk factors for cervical squamous carcinoma could also be not taking a Pap test at all.

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INVITED LECTURES ABSTRACTS

L24

Cytology of pleural effusions

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The pleural space normally contains between 7 and 16 ml of fluid. Any accumulation of fluid in the pleural space is the result of an increased production exceeding the rate of fluid removal . Pleural effusion as initial manifestation in a patient without accompanying symptoms is a major diagnostic problem. A large number of diseases may be the cause of pleural effusion. (1)The most common benign etiologies are infections (bacterial, tuberculosis, fungal, viral, parasitic), congestive heart failure, pulmonary embolism, myocardial infarction, cirrhosis, nephrotic syndrome, collagen vascular disease, pancreatitis, and trauma. Although most patients with a malignant effusion have a known history, a positive effusion may be the first indication of an unsuspected malignancy. In patients without a known primary site, clinical features such as age, sex, and the serous cavity involved can help narrow down the diagnostic possibilities. The most common primary sites for malignant pleural effusions in male patients are in descending order of frequency lung, lymphoma/leukemia, gastrointestinal (GI) tract, and genitourinary tract. In female patients with a malignant pleural effusion, the most common tumor types reported in descending frequency are breast, female genital tract, lung, lymphoma/leukemia, and GI tract. There is no single cytomorphologic feature to differentiate metastatic malignancy from reactive mesothelial cells . However, several cytologic findings including the low power pattern arrangement of the cells and the high power morphologic features combined together should be helpful for the correct diagnosis. Perhaps the most important cytologic feature to keep in mind is the range of appearance of reactive mesothelial cells. Reactive mesothelial cells can occasionally appear very atypical and therefore they could be a major pitfall for a false positive diagnosis of malignancy. Mesothelial cells are oval to round in shape with prominent cell borders. In reactive and hyperplastic mesothelium, the mesothelial cells aggregate in monolayers and 3-dimensional papillary groupings; numerous scattered mesothelial cells are also present. The nuclei of mesothelial cells are oval to round with a prominent nuclear membrane. Mesothelial cells can also present cytoplasmic vacuoles, but in contrast to the mucin vacuoles of many adenocarcinomas, the vacuoles tend to be paranuclear, small, and indistinct; sometimes mesothelial cells tend to look like histiocytes or macrophages, secondary to degeneration. The recognition of foreign cells is the most important cytologic feature for making a diagnosis of metastatic malignancy. Therefore, appreciation of a dual population consisting of foreign-appearing cells in a background of reactive mesothelial cells is extremely helpful, although occasionally a metastatic neoplasm can present with a uniform single population of malignant cells. The cellular arrangement at low and high power can help to suggest the primary site; Malignant cells can be arranged in papillary clusters, cannonballs (tightly cohesive spherical cell clusters with an almost perfectly round contour), clumps, or gland-like acinar groupings. When papillary clusters are present, the diagnostic possibilities include ovarian epithelial neoplasms, mesothelioma, and papillary thyroid carcinoma; other diagnostic possibilities for papillary clusters are colon carcinoma, lung adenocarcinoma, renal cell carcinoma, urothelial carcinoma, pancreatobilliary carcinoma, and embryonal carcinoma. If three-dimensional clusters or cannonballs are present, the diagnostic

possibilities include breast cancer, lung cancer, malignant mesothelioma, ovarian epithelial neoplasms, and mesothelial hyperplasia.

Acinar or gland formation should suggest lung, colon, and gastric adenocarcinoma, ovarian epithelial neoplasms, mesothelial hyperplasia, mesothelioma, breast carcinoma, renal cell carcinoma, pancreatobiliary carcinoma, prostate carcinoma, and endometriosis/ endosalpingiosis. When small malignant single cells are present in effusions, diagnostic possibilities include small cell carcinoma, lymphoma, lobular or ductal carcinomas of the breast, gastric adenocarcinoma, and a variety of small round cell tumors of childhood. A linear arrangement of cells with nuclear molding is characteristic of lobular carcinoma of the breast and small cell carcinoma. Malignant mesothelioma is an extremely challenging diagnosis in effusion cytology as they can have large cellular aggregates although single cells are usually also present and may predominate. Another important cytologic feature associated with malignant mesothelioma is lack of a foreign population.

Immunocytochemistry (ICC) can often be helpful in determining the site of origin of malignant cells present in an effusion cytology specimen. ICC can be performed in smears, cytospins, and ThinPrep, but the optimal cytologic specimen for ICC is the cell block. The antibodies panel should be chosen based on the differential diagnosis generated by the cytomorphologic findings, as well as the clinical features of site (pleural, peritoneal, or pericardial), age and gender. The appropriate panel should also include both positive and negative markers. A pattern recognition approach integrated with clinical findings can provide the diagnosis or at least suggest the etiology in most cases; Effusions can be the initial presentation of an occult malignancy, although more often, the patient will have a known history of cancer. Although adenocarcinoma is the most common histologic type, a variety of other malignancies can cause effusions and ancillary studies such as ICC may represent an important aid in the diagnosis of challenging cases (2-3). Our record of cases seems being particularly reliable, as in locating tumor of unknown sites refers to cytoautoptical comparison (an average of 2000 autopsies per year are performed every year at our department).

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INVITED LECTURES ABSTRACTS

L25

Molecular pathology of gastrointestinal lymphomas: current clinical practice and what to expect in the future ?

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The gastrointestinal (GI) tract is the most common site of primary extra-nodal lymphoma, accounting for 30-50% of cases. This is not surprising since the GI tract contains more native lymphoid tissue than that collectively present in all other major lymphoid organs. All categories of lymphoma that typically arise in the lymph nodes may also arise in the GI tract. GI lymphomas are often first detected in endoscopic biopsies. However, accurate diagnosis and classification often pose problems for the pathologist for several reasons. First, the small size of the endoscopic biopsy sample limits tumour load and pattern recognition. Second, the lymphoid tissue of the GI tract may show intense reactive hyperplasia which may mimic lymphoma. Third, in a fashion similar to cutaneous lesions, those in the GI tract may be visualized and biopsied at a very early phase in their development when differentiation into neoplasia may be incomplete. Some forms of immune response actually pass through a poorly defined transition into lymphoma. Examples of such 'dysplasia' of the immune system of the GI tract include *Helicobacter pylori (H. pylori)* gastritis, coeliac disease and multicentric lymphoid hyperplasia associated with underlying immunodeficiency. With ever increasing endoscopic scrutiny of the gut by gastroenterologists, it is not surprising that the frequency of these indeterminate cases seems to be growing. In combination with careful clinical correlation and conventional microscopic analysis, selective immunohistochemical studies and molecular pathology currently constitute the most powerful methods in the pathologist's effort to recognize and classify GI lymphomas accurately.

This lecture covers molecular biology and molecular cytogenetic aspects of GI lymphomas, that not only lead to new insights into the biology of these tumours, but also provide the pathologist with diagnostic tools and potential prognostic factors.

GI **mucosa associated lymphoid tissue (MALT) lymphoma** accounts for 5-10% of all GI malignancies and at least 50% of all gastric lymphomas, making it the most frequent lymphoma of the GI tract [1]. Gastric MALT lymphoma is clearly linked to *H. pylori* infection. Sustained antigenic stimulation by *H. pylori* not only triggers a polyclonal B-cell proliferation, but also attracts neutrophils to the site of inflammation, with the subsequent release of reactive oxygen species (ROS). The latter are genotoxic and cause a wide range of genetic abnormalities. Moreover, prolonged proliferation of B-cells induced by chronic inflammation may also increase the risk of DNA damage, like double-strand DNA breaks, due to the intrinsic genetic instability of B-cells during somatic hypermutation and class switch recombination. Several of these genotoxic events in MALT lymphomas have been identified, being translocations t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21) and t(3;14)(p13;q32), resulting in *API2-MALT1, IGH-BCL10, IGH-MALT1* and *IGH-FOXP1* rearrangements respectively, with t(11;18)(q21;q21) being the most frequently occurring anomaly in GI lymphomas [2]. Remarkably, the genes targeted by 3 of these translocations participate in one and the same pathway resulting in the activation of nuclear factor κB (NF-κB), which is a key transcription factor in immune responses.

NF-κB regulates the expression of a number of survival- and proliferation-related genes in B-cells, and as such, its constitutive activation results in uncontrolled B-cell proliferation and thus lymphomagenesis [3]. As is the case with all GI B-cell non-Hodgkin lymphomas, demonstration of monoclonal rearrangement of the immunoglobulin heavy chain *(IGH)* gene and/or immunoglobulin light chain (*IGK* or *IGL*) gene by polymerase chain reaction (PCR) is an important tool in the hands of the pathologist to prove B-cell clonality and as such confirm lymphoma diagnosis. However, to date, there is no specific immunohistochemical marker for MALT lymphomas. Nevertheless, the presence of recurrent genetic abnormalities may facilitate and/or confirm the diagnosis of a MALT lymphoma : presence of t(11;18)(q21;q21) in tumour tissue, either fresh-frozen or paraffin-embedded, can be detected by cytogenetic analysis (karyoptyping), reverse transcription-PCR (RT-PCR) and fluorescence in situ hybridisation (FISH). One or more of these techniques are now routinely performed for several cancer screening tests in most laboratories. Although the presence of t(11;18)(q21;q21) may facilitate and/or confirm the diagnosis of a MALT lymphoma, current guidelines do not recommend a routine screening for the t(11;18)(q21;q21) once the diagnosis of a gastric MALT lymphoma is established : all patients with *H. pylori*-positive gastric MALT lymphoma should undergo eradication therapy, regardless of their t(11;18)(q21;q21)-status and moreover, the presence of a t(11;18)(q21;q21) in a MALT lymphoma does not exclude progression to a DLBCL as shown by new data. GI MALT lymphoma is an indolent lymphoma with a 5-year survival rate of approximately 95%. However, once transformation to a more aggressive diffuse large B-cell lymphoma (DLBCL) occurs, the 10-year survival-rate drops to less than 50%. The mechanism by which an indolent MALT lymphoma transforms into an aggressive DLBCL are not yet know, although studies identified the transcription factor FOXP1 as a key molecule in this process [4].

Although GI **follicular lymphoma** is usually secondary to nodal disease, it may present as a GI lesion and, therefore, must be distinguished from MALT lymphoma, particularly those with colonization of the follicle centre by tumour cells [5]. In contrast to colonized reactive follicles, the neoplastic germinal centre cells of follicular lymphoma usually express the anti-apoptotic protein BCL2 as a result of *BCL2* gene rearrangement by the translocation t(14;18)(q32;q21). As such, an often critical point in the differential diagnosis is also the demonstration of this t(14;18)(q32;q21))/*IGH-BCL2* in follicular lymphoma by either RT-PCR or FISH, as opposed to the presence of t(11;18)(q21;q21), t(3;14)(p13;q32), t(1;14)(p22;q32) or t(14;18)(q32;q21)/*IGH-MALT1* in MALT lymphoma.

Mantle-cell lymphoma is another spectrum of low- to high-intermediate grade B-cell neoplasms with a striking tendency for involvement of the GI tract [6,7]. It may present as the spectacular phenomenon of lymphomatous polyposis which is characterized by the presence of multiple polyps along the GI tract [8]. Mantle cell lymphoma predominantly occurs in older males and was originally described by Lennert and coworkers in Kiel as 'centrocytic' lymphoma, and more vaguely in the USA as 'intermediately differentiated lymphocytic lymphoma'. This distinctive B-cell lymphoma features CD5 expression in combination with cyclin D1 overexpression, the latter being a consequence of the BCL1 translocation which juxtaposes the immunoglobulin encoding regions of the heavy chain region on chromosome region 14q32 next to the *PRAD1* oncogene region on chromosome region 11q13.

While recognition of mantle cell lymphoma is straightforward in a case of full-blown lymphomatous polyposis, in earlier cases with more subtle endoscopic findings the early

partial involvement of lymphoid aggregates by the neoplastic mantle cellularity is often difficult. Fortunately, immunohistochemical demonstration of nuclear staining for cyclin D1 is usually conclusive, as well as molecular-genetic tests (karyotyping, FISH or RT-PCR) that investigate the presence of the translocation t(11;14)(q13;q32).

Post-transplantation lymphoproliferative disorders (PTLD) represent the most spectacular and challenging cases for clinician and pathologist alike, occurring as explosive proliferations in the setting of immunosuppressed transplant recipients. Such processes often arise in extranodal sites, in particular within the organ transplants themselves, or in the GI tract. Although a small percentage of these proliferations are microscopically recognizable as benign ('plasma cell hyperplasia') or malignant ('anaplastic plasmacytoma'), the majority have a highly proliferative polymorphous appearance which suggests true neoplasia, but which often responds to diminished immunosuppression (sometimes in combination with anti-CD20 immunotherapy) [9-11]. The pathologist's recognition of this syndrome, as distinct from conventional lymphoid neoplasms, depends upon the clinical history and microscopic identification of telltale clues, including geographical necrosis, polymorphism, abundant dispersed apoptotic cells, variable plasmacytic maturation, and the presence of occasional Reed–Sternberg-like multilobated immunoblasts reminiscent of acute infectious mononucleosis [9]. Because almost all such proliferations are EBV positive, as is demonstrable with immunostains for latent membrane protein or with *in situ* hybridization for EBV-encoded RNA, these markers may prove extremely useful in even small biopsy samples. Clonality studies are not predictively useful, since monoclonal proliferations, defined either by molecular probes or immunoglobulin light chain restriction by immunophenotype, are not necessarily malignant, i.e. irreversible.

Burkitt and **'Burkitt-like' lymphomas** are among the most aggressive, most proliferative of all human neoplasms and were collectively designated as 'small non-cleaved cell type' in the Working Formulation and as Burkitt or 'Burkitt-like' in the World Health Organization (WHO) classification [12]. The classic presentation is as an obstructing tumour of the terminal ileum arising in a young patient, presumably arising within Peyer's patches, but primary GI tract sites elsewhere in small or even large intestine occur. Because of the extremely high kinetics of this tumour it is particularly vulnerable to autolysis, so that poorly fixed tissues can simulate the appearances of low-grade (small lymphocytic) lymphomas. In classic Burkitt lymphoma nuclei are relatively uniform, medium (not small) sized with thick chromatin rims and multiple prominent nucleoli. There is deeply basophilic cytoplasm with clear vacuoles representing lipid droplets. Although the 'Burkitt-like' variant was proposed as a provisional type in the Revised European-American Lymphoma (REAL) classification of 1994, the trend (e.g. in the new WHO system) has been to establish this as an entity, despite poor reproducibility [12]. This entity consists of cases with greater pleomorphism than Burkitt type proper but with all other features such as extremely high proliferative rate (at least 90% nuclear staining for Ki67). These neoplasms are endemic in settings with neonatal Epstein–Barr virus (EBV) exposure and malaria, and in HIV-positive individuals, but also occur sporadically without EBV association in high socioeconomic settings without immunodeficiency.

In contrast to 'Burkitt-like' lymphoma which represents an extremely heterogeneous disease on the genetic level, true Burkitt lymphoma is a homogeneous disease by gene expression profiling and, almost by definition, it is associated with *C-MYC* gene translocation [13]. This gene is found at 8q24 and the most common variant of the translocation is the t(8;14)(q24;q32)

which places *C-MYC* under control of the *IGH* gene enhancer. A variant of this, a three-way translocation, t(8;14;18), has also been identified as well as the rare variants t(2;8)(p12;q24) and t(8;22)(q24;q11), which involves *C-MYC* and the immunoglobulin kappa and lambda light chain genes respectively. Demonstration of *C-MYC* rearrangement by either karyotype, FISH or RT-PCR is an important tool in the diagnosis of true Burkitt lymphoma.

Although far less frequent in comparison to B-cell non-Hodgkin lymphomas, a variety of T-cell lymphomas may also arise in the GI tract, such as extranodal NK/T-cell lymphoma, γδ T-cell lymphoma, anaplastic large cell lymphoma (ALCL) and **enteropathy-associated T-cell lymphoma (EATL)** [12]. The latter is the most studied T-cell lymphoma of the GI tract. It usually presents as a tumour composed of large lymphoid cells with an inflammatory background, and is associated with celiac disease. However, in 20% of cases, EATL is composed of monomorphic medium-sized cells and only rarely arises in the background of celiac disease (so called type II EATL) [14]. Tumour cells of EATL express CD3, CD5, CD7 and CD103, as well as the cytotoxic granule markers TIA1 and perforin. In addition, EATL tumour cells are mostly CD8 and CD56 negative, while type II EATL tumour cells are mostly CD8 and CD56 positive [15]. The application of PCR to investigate the presence of monoclonal T-cell receptor rearrangements proofs very useful in the diagnosis of an EATL and other intestinal T-cell lymphomas. However, no other molecular-genetic test are currently available as most intestinal T-cell lymphomas harbour very complex karyotypes. Exception to the rule is ALCL, where rearrangement of the *ALK1* gene by the translocation t(2;5)(p23;q35) can be demonstrated by either cytogenetic analysis, PCR or FISH [16].

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INVITED LECTURES ABSTRACTS

L26

The neuropathology of infant subdural haemorrhage

W. Squier

Dural and cerebral immaturity

As far as intracranial anatomy and physiology are concerned, the human infant does not catch up with his primate cousins until well into the second year of life. Evolution of a large head has meant a number of developmental compromises; a major one being born months too soon in order that the head can pass through the narrow pelvis necessitated by upright gait. The human brain triples in weight in the first year of life, largely by the acquisition of myelin. To accommodate this rapid brain growth the skull remains thin and pliable with open sutures and the vascular supply must adapt. All blood draining from the brain passes through the dura, and the venous sinuses undergo remodelling during the first years of life(1)(Browder 1975).

A further adaptation to postnatal life is required by the transformation of the fetus from an aquatic to a terrestrial, air-breathing mammal. This is associated with a 30% reduction in body and brain water content in the first months of life when the arachnoid granulations (AG), traditionally believed to be responsible for CSF resorption, are absent or immature. The brain must have alternative means of fluid uptake, and it is now considered that a major route of CSF absorption is via the parenchyma and by cranial, and particularly spinal, nerve roots.

The purpose of this talk is to describe some of our studies on how the infant brain and dura may be equipped to handle fluid and how immaturity may lead to vulnerability to dural bleeding and brain swelling.

Studies of the dura from fetal life to the 9th decade have confirmed the presence of a system of unlined fluid channels (Squier 2009) (2) , first described in 1875 but largely ignored until 1996 (Fox 1996)(3). These channels are increasingly evident after the first 6 months of life. Conversely, intradural bleeding becomes less common at this time, which corresponds to the development of arachnoid granulations.

As long ago as 1910 Cushing suggested that the AG function as valves and Welch (1960) (4) showed this to be so, suggesting that they prevent reflux of blood from the dural sinuses into the subarachnoid space. In the absence of AG raised pressure within the dural sinuses may lead to dural bleeding, which is common in the young infant (Cohen 2008)(5).

Water handling in the infant dura and brain

Aquaporins are transmembrane pore proteins that selectively transport water molecules (6). Aquaporin 1 is highly expressed in the choroid plexus where it is thought to play a major role In CSF production. AQP1 is expressed in systemic, but not in brain, endothelial cells. Its absence from brain endothelia is believed to be a related to the highly selective nature of the blood brain barrier. We have found that AQP1 is expressed in the endothelium of blood vessels in the dura but not the brain, and in some cases it is expressed in dural fibroblasts. The importance of AQP1 to normal fluid homeostasis is well established, and its presence in

the dura raises important questions with regard to the role of the dura in water homeostasis of the brain.

With respect to the brain, water tends to accumulate in the subcortical white matter in the young infant, leading to subcortical leucomalacia. There are a number of possible explanations for this; the subplate zone, which contains hydrophilic proteins does not involute until several months after birth (7), there may be immaturity of the venous drainage of the brain and there may be selective developmental expression of aquaporins. A number of cases will be shown where hypoxia and brain swelling in young infants was associated with upregulation of aquaporins 1 and 4 in subcortical astrocytes. Clefts in this zone in infants below 5 months of age have been called subcortical contusions and ascribed to trauma (Lindenberg 1969) (8). Our observations indicate that this terminology may be inaccurate and these clefts may result from fluid accumulation in brain swelling from atraumatic as well as traumatic causes.

The natural history of Infant Subdural Haemorrhage

Infant subdural haemorrhage tends to form a thin, bilateral film. Thick space-occupying blood clots are more common after the first year of life (Geddes 2001)(9;10). Autopsy studies suggest that thin film bleeding may originate in the dura; intradural bleeding is common in the infant and, by microscopy, blood is frequently seen to escape onto the subdural surface, forming a film between the dura and the arachnoid barrier layer. Blood leaking from dural fluid channels may be mixed with fluid, which would explain why these bleeds are frequently unclotted and spread widely. It is common to find blood in the spinal subdural compartment in the presence of intracranial SDH. The spinal blood is usually posterior and at the most dependent levels of the cord; this distribution suggests gravitational redistribution of blood in the subdural compartment. MRI studies have shown spinal blood in 50% of infants with intracranial SDH (Koumellis 2008)(11).

Once blood escapes from the vessels of the dura an inflammatory and reactive healing process is set into motion. New vessels can be seen sprouting into the clot within three days. Red cells break down and haemosiderin is demonstrated with Perl's stain after 2-3 days. Macrophages may be numerous. Fibroblasts grow into the membrane and capillaries become larger, with thin walls and wide lumens. These reactive processes are well demonstrated with special stains, particularly endothelial markers (CD 31, CD 34 and Factor VIII) and the macrophage marker CD68. It is helpful to use at least some of these stains as a thin membrane may not be easily distinguished from normal dura. The age of a bleed may be roughly assessed by reference to the thickness and vascularity of the membrane (Menkes *Child Neurology* 2006 fig 9.10), but this is not precise. Little information is available for the infant dura.

Subdural membranes may persist as a thin layer of brown, patchy discolouration of the dura many months after birth (Keeling in *"Paediatric and Forensic Medicine and Pathology"* 2009 p 218). Microscopic examination of healing subdural membranes almost always demonstrates small foci of rebleeding of different ages, indicating vulnerability of the thin vessels. Fresh bleeding into healing membranes is very common in babies who are ventilated for the last weeks of life, indicating that bleeding may result from swings of intravascular pressure during ventilation or brainstem death.

Both dural sinus thrombosis and cortical venous thrombosis can present with SDH. The precise mechanism by which SDH is produced in these circumstances is not well understood (12).

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INVITED LECTURES ABSTRACTS

L27

Do Gastrics Lymphoid infiltrates remain a challenge for Pathologists?

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Chronic inflammation of gastric mucosa, usually devoid of organized lymphoid tissue, may be due to a variety of causes, the major aetiology being the colonization by *Helicobacter pylori* (*Hp*). Lymphoid follicles are a regular finding in *Hp* infection and can even persist for years after successful *Hp* eradication. Gastric MALT (mucosa-associated lymphoid tissue) lymphoma is closely related with *Hp* infection and represents probably a multistage process, from gastritis to low-grade or high-grade lymphoma.

For the differential diagnosis of gastric MALT lymphoma and reactive inflammation, Wotherspoon *et al* proposed a histomorphological scoring widely used (table). This score contains two intermediate categories, grades 3 and 4 (favoring respectively reactive or neoplastic infiltrate), meaning that additional tests need to be realized to discard or confirm lymphoma. In this molecular era, the impact of genetic studies (Ig clonality by PCR and/or MLT translocations and chromosomal aberrations such as trisomy 3 by fluorescent in situ hybridization) on daily practice is still under debate.

We will present our local experience (clinique Saint-Luc, Brussels). More than 14,000 biopsies containing "gastric lymphoid infiltrate" were retrieved from the files from January 1, 2004 to December 31, 2008 (5 years). Amongst them, 124 (0.9 %) were ambiguous or lymphomatous, and concerned 77 patients. 34 patients (44%) suffered from frank lymphoma, with a predominance of MALT lymphoma (50%) but also including diffuse large B cell lymphoma (29%), mantle cell lymphoma (15%), Burkitt-like lymphoma (3%) and follicular lymphoma (3%). The remaining 43 patients were affected by Wotherspoon grade 2 to 4 gastritis. Seventeen (40%) out of these were followed later, at least once and lymphoma was present in further biopsies in 6 cases (35%), with no or little association with *Hp* (2/6) at the first biopsy.

In conclusion, the diagnosis of gastric lymphoma remains a diagnostic challenge for pathologists, especially for cases with ambiguous Wotherspoon grades. Even in the molecular era, histology, immunohistochemistry and clinical follow-up should be considered as the gold standard for the diagnosis. Moreover, pathologists must keep in mind that aggressive lymphomas e.g. diffuse large B cell lymphoma, mantle cell lymphoma or Burkitt lymphoma can also involve the stomach.

Table: Histological scoring of lymphoid infiltrations in the stomach according to Wotherspoon.

score	Diagnosis	Histological features
0	normal	Scattered plasma cells
1	Chronic active gastritis	Score 0 + small clusters of lymphocytes
2	Chronic active gastritis with florid lymphoid follicle formation	Score 1 + lymph follicles
3	Suspicious lymphoid infiltrate, probably reactive	Score 2 + surrounded by lymphocytes that infiltrate diffusely into the lamina propria
4	Suspicious lymphoid infiltrate, probably lymphoma	Score 3 + small groups into the epithelium
5	MALT lymphoma	Score 3 + prominent lymphoepithelial lesions

INVITED LECTURES ABSTRACTS

L28

Neuroradiologic findings in young children with subdural haemorrhage

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Abstract

Paediatric neurotrauma is a serious and potentially life threatening condition with a reported morbity of 31-45% and mortality of 6-26% (1-3). In the paediatric age group neurotrauma is one of the major causes of death (exact figures are lacking due to assumed underreporting).

Radiology plays an important, if not vital, role in the detection of intracranial pathology. The mainstay of imaging, especially in the emergency setting, consists of Computed Tomography (CT) and the lecture will be focused on neuroradiological findings on this modality. However, ultrasonography and Magnetic Resonance Imaging (MRI) are used as well and their use and limitations will be discussed. Special attention will be given to the (dis)ability to date intracranial pathology in children.

Subdural haemorrhage in young children can be the result of: birth-trauma, benign enlargement of the subarchnoid space (BESS), accidental trauma, and Inflicted Traumatic Brain Injury (ITBI). ITBI is currently the correct term for what formerly was known as 'shaken baby syndrome'. This phrase was originally coined by John Caffey (1895-1978), a paediatric radiologist in Boston, who was one of the first to recognize the now classical triad of intracranial trauma, retinal haemorrhages and ribfractures (4).

As in young children ITBI should, in nearly all cases, be part of the differential diagnosis emphasis will also be placed on the role of the (paediatric) radiologist in the clinical work-up of these patients.

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INVITED LECTURES ABSTRACTS

L29

Pathologist inut in a one stop clinic for breast lesions

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We have been instrumental in implementing a one stop multidisciplinary breast clinic at the Institut de cancérologie Gustave Roussy since 2004. Our team, specialized in senology, consists of a surgeon, a medical oncologist or radiotherapist, a radiologist and a cytopathologist. The aim is to deliver weekly, within the same day and usually in a series of 20 patients, as many as possible definitive diagnoses based on the on site examination of fine needle aspiration cytology specimens. On site examination of breast nodular samples, performed by the cytopathologist and most frequently under the guidance of ultrasonography, dramatically lowers the number of unsatisfactory specimens. This procedure is also highly effective and comparable with the results of core needle biopsy in terms of efficiency. In addition, core needle biopsy may be performed immediately when required i.e. if cytology is unsatisfactory, suspicious or discordant with clinicoradiological findings. This very effective method of triage for patients with breast nodular lesions is also cost effective and useful for acquiring, with the patient informed written consent, cytological material for ancillary studies using conventional or more sophisticated methods applicable to minute samples.

INVITED LECTURES ABSTRACTS

L30

Angiogenesis / Anti-angiogenic therapy of glial brain tumours.

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Gliomas form a heterogeneous group of tumors of the central nervous system, encompassing many different histological types and malignancy grades. Most gliomas (esp. astrocytic, oligodendroglial, and mixed/oligoastrocytic tumors) are characterized by diffuse infiltrative growth of tumor cells in the neuropil. The most malignant (astrocytic) glioma, i.e. glioblastoma, is unfortunately also by far the most common. In gliomas of high grade malignancy, prominent, often "glomeruloid" microvascular proliferation and necrosis emerge. These changes are in fact used as histopathological criteria to diagnose high-grade malignancy in glial tumors and are generally spatially and temporally related. Hypoxia in gliomas may be caused by more compact tumor growth or after thrombosis in tumor blood vessels. Hypoxia induces increased expression of hypoxia inducible factor 1α (HIF-1α) and vascular endothelial growth factor (VEGF), two factors that play a crucial role in the induction of angiogenesis in tumors. Alternatively, angiogenesis in gliomas may be driven by constitutive production of VEGF-A by the tumor cells. Interestingly, even though glioblastomas may show an extreme angiogenic response, extraneural metastases of these tumors are extremely rare.

Magnetic resonance imaging (MRI) is now the gold standard for defining brain tumor "anatomy" in a clinical setting. Low grade diffuse astrocytic gliomas generally do not show enhancement when using the contrast-agent Gadolinium-DTPA. The absence of contrast-enhancement in these tumors can be explained by incorporation ("coöption") of preexistent microvessels with only limited changes to the blood-brain barrier (BBB) and lack of neovascularization. The fact that high grade gliomas show contrast-enhancement on MRI scans indicates that in such regions the BBB in preexistent and/or in newly formed microvessels is disrupted. Stimulated by VEGF and other growth factors (incl. FGF, PDGF, TGF), the capillaries in brain tumors are abnormally formed, and endothelial cells become hyperplastic, show increased transendothelial transport, loss of tight junctions, and formations of fenestrations, resulting in increased BBB permeability. However, also in high grade gliomas the periphery of the diffuse infiltrative neoplasms often lacks contrast enhancement and accurate radiological assessment of response in individual patients to different therapeutic regimens is notoriously difficult. Correlation of histological sections of glioblastomas with radiological images revealed that tumor cells were often present several centimeters outside the enhancing area.

The diffuse infiltrative growth pattern of these glial tumors is a major obstacle for curative treatment as it virtually precludes complete tumor removal, while the potential of radiotherapy is limited because of detrimental side effects to the brain.

Chemotherapy is so far not very effective because of chemoresistance of the glioma cells and/or the presence of the BBB which impairs adequate penetration of potentially promising cytostatic drugs in the tumor tissue, especially in areas in which the original tissue architecture is relatively preserved.

As VEGF-A is now generally considered as the main inducer of physiological and pathological angiogenesis, the mode of VEGF action has become the focus of attention for the development of angiogenesis inhibitors (AIs). Bevacizumab (Avastin), a neutralizing anti-VEGF-A antibody, was the first FDA-approved anti-angiogenic compound. Many VEGF and VEGFR targeting antibodies and VEGFR tyrosine kinase inhibitors (TKIs) are now in clinical testing and efficacy and toxicity data are becoming available almost daily. Notably, many of these studies have not been performed in patients with primary brain tumors. Up till now, neurological side-effects in patients with solid tumors have been described predominantly for TKIs and far less for antibodies targeting VEGF and VEGFR. This might be due to the fact that (in contrast to TKIs) the antibodies are not able to cross the intact BBB. The exact effect of different classes of AIs on the BBB remains to be elucidated.

Because of the florid angiogenic response in glioblastomas, these tumors have since long been considered as good candidates for anti-angiogenic therapy. Originally, the general belief was that application of AIs would simply inhibit growth of solid tumors by reducing vascularization and vessel permability, thereby depriving the tumor tissue from oxygen and nutrients. Indeed, VEGF-inhibition results in potent anti-tumour effects in a variety of tumor xenograft models in nude mice. Such findings have led to clinical trials in glioma patients with different AIs (both VEGF antibodies and TKIs for VEGFR), either as monotherapy or combined with cytostatic agents. Unfortunately, in the clinical setting monotherapy with AIs has not yet met initial expectations.

The underlying cause for the discrepancy between the effects of AIs in preclinical tumor models and clinical trials is a matter of debate. In mice carrying rapidly growing subcutaneous tumors, the blood vessels are predicted to be in a more or less synchronized stage of development. In patients, tumors are generally heterogeneous with areas of active angiogenesis co-existing with regions in which the vasculature has already matured. It is conceivable that these latter regions will be resistant to anti-angiogenic therapies. Another important notion is that especially in vessel dense-tissues such as lung, liver and brain, tumors may grow without angiogenesis by incorporating pre-existent vessels. Indeed, many vessels in diffuse infiltrative astrocytic tumors may be incorporated rather than newly formed. In the most extreme example of diffuse infiltrative glioma growth in the brain, i.e. gliomatosis cerebri, combined quantitative and immunohistochemical analysis of the brain microvasculature suggested a complete lack of angiogenesis and absence of increased BBB permeability.

On the one hand, application of AIs may inhibit tumor growth by reducing vascularization, vessel permeability, and thereby deprive the tumor tissue from oxygen and nutrients. This may not only be a positive effect, as hypoxic tumor cells tend to escape irradiation, and drug delivery may be impaired by the decreased number and permeability of the tumor vessels. Alternatively, AI-induced normalization of the microvasculature may enhance the effect of radiation and chemotherapy via reduced interstitial pressure and more efficient delivery of chemotherapeutics and oxygen to the tumor tissue. In diffuse infiltrative glial tumors, normalization of the microvasculature by AIs could imply restoration of the BBB.

INVITED LECTURES ABSTRACTS

Using a genotypically and phenotypically relevant, orthotopic model of human glioblastoma in the mouse brain we recently found a dose-dependent effect in the form of increased hypoxia, necrosis, inhibition of glomeruloid microvascular proliferation of the AI vandetanib (a tyrosine kinase inhibitor with specificity against VEGFR2, Epidermal Growth Factor Receptor (EGFR) and Rearranged during Transfection/RET) in more compact areas, whereas the diffuse infiltrative parts in this model were not notably affected. Moreover, combination treatment with temozolomide and vandetanib had an adverse effect on chemotherapeutic efficacy in this model. Furthermore, in this model vandetanib restored the functionality of the BBB, thereby preventing visibility of tumors using Gd-DTPA-enhanced MRI. A similar effect was found for other AIs (Avastin; Sutent, a TKI of VEGFR, PDGFR, RET, KIT and flt-3). Finally, in some experimental glioblastoma models the blockade of vascular changes by anti-VEGF therapy resulted in increased vessel coöption by the tumor.

Still, AIs may be beneficial for glioblastoma patients, exerting their effect in more cellular, compact areas and reducing brain edema. Indeed, follow-up scans of glioma patients that were treated with bevacizumab revealed reduced vasogenic brain edema and (esp. in tumors with heterogeneous rather than solid enhancement) reduced contrast enhancement of the neoplasms. It is, however, becoming increasingly clear that AI-induced normalization of the glioma microvasculature may result in overestimation of the therapeutic efficacy. Improved imaging modalities are thus needed to monitor response to AIs. Furthermore, esp. since the results of monotherapy with most AIs in clinical trials have been disappointing so far, it is likely that AIs must be combined with other therapeutic modalities in order to obtain a significant impact on patient survival.

Optimalization of dosage and scheduling of AIs in combination with other therapeutic modalities will help to augment rather than antagonize the response to chemotherapy. Up till now, many promising compounds for the anti-angiogenic treatment of glioblastoma patients are being tested in non- or less relevant model systems (e.g. in cell cultures or heterotopic tumor models in which the brain microenvironment is completely lacking), or directly in clinical trials. Ideally, however, testing of promising compounds and identification of optimal therapeutic regimens should be performed in genotypically and phenotypically relevant, orthotopic animal models, thereby protecting glioma patients from negative side effects of regimens that are unlikely to provide any benefit.

ORAL PRESENTATIONS

ORAL PRESENTATIONS LIST

ORAL PRESENTATIONS ABSTRACTS

O 01

Nucleic acid-sequence based amplification assay for HPV mRNA detection and typing: evidence for DNA amplification.

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In productive human papillomavirus (HPV) infections the expression of the viral oncogenes E6 and E7 is tightly regulated, whereas in high grade lesion and cancer E6 and E7 are expressed throughout the thickness of the cervical epithelium. Therefore, E6/E7 mRNA has been proposed as a more specific marker for cervical dysplasia and cancer than HPV DNA. Multiplex nucleic acid sequence-based amplification (NASBA) assays, which utilise molecular beacon probes for real-time detection and typing of E6/E7 mRNA from HPV types 16, 18, 31, 33 and 45 are commercially available. However, unexpected amplification of double-stranded DNA by NASBA has been reported, which indicates the necessity of verifying the origin of a NASBA signal when specific RNA detection is the objective.

This study evaluated the RNA-specificity of NASBA-based HPV detection using HPV DNA plasmids (HPV16, 18, 31, 33, 45) and nucleic acid extracts of several cell lines, which were systematically subjected to enzymatic treatments with DNase and RNase.

HPV plasmids dilutions ($1x10^6$ to $1x10^0$ copies/µl) and nucleic acid extracts (total DNA, RNA-free DNA, total RNA, DNA-free RNA) of unfixed and fixed (PreServCyt® and SurePath®) HaCaT, HeLa and CaSki cells were tested with the NucliSENS EasyQ® HPV test.

The RNA-free DNA extracts of HeLa and CaSki cells could be amplified by HPV18 and 16 NASBA, respectively. Fixation of the cells did not influence NASBA. All HPV plasmids could be detected with NASBA. Based on the plasmid dilution series, a lower detection limit of $5x10^3$ HPV DNA copies could be determined.

Our study identified viral double-stranded DNA as possible target for NASBA-based HPV detection. The differences in diagnostic accuracy between the NASBA-based tests and conventional HPV DNA detection assays do not seem attributable to the more specific amplification of viral mRNA, but to the limited type range and the lower analytical sensitivity for HPV DNA.

ORAL PRESENTATIONS ABSTRACTS

O 02

What's new about HPV and HNSCC?

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Introduction:

Human papillomavirus (HPV) infection has been well established as a risk factor for developing head and neck squamous cell carcinoma (HNSCC), independent of tobacco and alcohol use. The detection of HPV in HNSCC has important clinical implications such as a better prognosis, better response to treatment and detection of disease persistance or early recurrence.

Aim:

To determine the prevalence of HPV associated HNSCC in our institute and to correlate it with immunohistochemical detection of p16.

Material and methods:

During 3 years (from June 2007 until December 2009), 170 biopsies of primary HNSCC localized in the oral cavity, including tonsils, oropharynx and larynx were prospectively enrolled for HPV detection by PCR. PanHPV (GP5+/GP6+) and specific HPV subtypes (6/11,16,18,30,31) were searched for on paraffin-embedded material.

During the first 18 months, immunohistochemistry for p16 Protein was applied on biospies which were at least panHPV positive. After May 2009, it was done prospectively.

Results:

In total, HPV was found in 57 cases out of 170, representing 33.5% of cases.

HPV 16 was the predominant subtype (16 cases, 28%), followed by HPV6/11 (14 cases, 24.6%). Four cases were positive for HPV18 (7%), one for HPV 31 (1.8%) and in 11 cases (19.3%) non of the studied subtypes could be detected.

In 11 cases, HPV co-infection was present (19.3%) with 8 combinaisons of HPV16 - 6/11, one HPV31 - 33 and two HPV18 - 6/11. p16 Protein was positive in 19 cases of the 57 HPV positive biopsies (33.33%), 15 were at least HPV16 positive, one was HPV18 positive and 3 cases were positive for panHPV only.

Conclusion:

One HNSCC out of 3 showed at least panHPV positivity by PCR investigation. Only one third of them showed p16 immunoexpression. The most frequent HPVsubtype detected in our population was HPV16 in 24 out of 57 cases.

O 03

Combined analysis of HPV-DNA, p16, p21 and p53 to predict prognosis in stage IV hypopharyngeal carcinomas

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Introduction:

Head and neck squamous-cell carcinomas (HNSCCs) are the sixth most common form of cancer worldwide. Biological markers are required to predict high-risk tumours to define patients in need of more aggressive treatment. The aim of this study was to examine the combined expression of p16, p21 and p53 with human papillomavirus (HPV) DNA detection, as adjunct molecular markers in predicting survival in patients with stage IV hypopharyngeal squamous cell carcinomas (HSCC).

Materials and Methods:

HSCC patients (n=75) were evaluated for p16, p21 and p53 by immunohistochemistry (IHC). HPV was detected by GP5+/6+ consensus PCR and subsequent typing by E6/E7 type-specific PCR.

Results:

HPV testing identified 82% of patients with high-risk (hr) HPV types. HPV 16E7 DNA was detected in 37 cases. Twelve patients presented multiple HPV infections. However, high risk (hr)-HPV tumors did not disclose significant correlation with the clinical data and the proportion of disease-free patients. p16 positive cases (n=7) were correlated with better prognosis because they were free of disease. However, this difference was not statistically significant.

Sixty four percent of the tumours were p21 positive. The 5-year disease free survival was 60% in p21+ tumors versus 70% in p21- tumors. Considering the proportion of disease-free patients, this difference was statistically unsignificant.

p53 overexpression was detected in 27 cases of 73 hypopharyngeal SCCs. In the HPV- group, 36% of tumours presented p53 overexpression whereas 33% of the hr-HPV+ group expressed p53. The 5-year disease free survival was 73% in p53- tumors versus 48% in p53+ tumors (p=0.008).

Conclusions:

In our series of stage IV HSCCs, the hr-HPV+ subgroup presented a similar prognosis to the HPV- subgroup. p53 overexpression is associated with worse prognosis.

O 04

Indoleamine 2,3-dioxygenase expression at the tumor invasion front is an independent negative prognostic factor in pT1-4N1Mx staged colorectal cancer

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Purpose:

Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-catabolizing enzyme that induces immune tolerance by modulating T cell responses. Carcinomas may actively create an immunosuppressive state via the expression of IDO. In this study, we examined a possible contribution of IDO on this phenomenon and investigated whether IDO expression has prognostic value in colorectal cancer.

Experimental design:

IDO gene and protein expression was investigated by quantitative PCR and Western blotting in three colon cancer cell lines in basal state and after stimulation with interferon-γ (IFN-γ). Semiquantitative immunohistochemistry was used to evaluate IDO expression in tissue microarray materials of 265 pT1-4N0-2Mx staged colorectal cancer resection specimens. Results were statistically related to clinical variables and correlated with the amounts of $CD3^+$ and $CD8^+$ T lymphocytes which were quantitatively evaluated using image analysis.

Results:

In vitro the expression of the IDO gene and protein was found to be dependent on IFN-γ stimulation. Using multivariate survival analyses, we identified higher IDO expression at the tumor invasion front as being an independent adverse prognostic factor in pT1-4N1Mx staged colorectal cancer. It was significantly associated with overall survival ($p = 0.001$) and with the development of metachronous metastases ($p = 0.018$). In contrast, IDO expression was not significantly associated with the presence of $CD3^+$ or $CD8^+$ T lymphocytes.

Conclusions:

Our results indicate that higher IDO expression at the tumor invasion front is involved in the progression of colorectal cancer and correlates with impaired clinical outcome, suggesting that IDO is an independent and reliable prognostic indicator for colorectal cancer.

O 05

KI-67 HOT-SPOTS DETECTION ON GLIOBLASTOMA TISSUE SECTIONS

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Introduction

Considering the great heterogeneity encountered in GBM tissue, the percentage of proliferative cells - evidenced by Ki-67 staining - is not able to fully describe the complexity of the cell proliferation patterns exhibited by these tumors. The aim of this study is to further describe Ki-67 expression by identifying and characterizing the regions of strong expression, also known as "hot-spots" (HS). This approach has already been applied by means of manual identification in low magnification images [1]. We propose a novel method to automatically identify such Ki-67 hot-spots based on hierarchical clustering (HC) techniques.

Materials and Methods

We applied standardized protocols for IHC staining and image acquisition in order to ensure valid immunostain characterization using image analysis [2]. As a result of the staining procedure, the Ki-67 positive nuclei appeared brown and the negative ones blue due to counterstaining. We then proceeded to a color deconvolution step to separate the brown staining from the blue coloration [3] followed by a segmentation of the nuclei. The clustering operation was then performed on 2D datasets consisting of the location of each stained nucleus. We tested 3 clustering criteria: Ward's criterion, Single Linkage criterion and Germini, a novel criterion based on the original CHAMELEON concept of considering local topology to cluster the data [4].

Results and conclusion

Ward's criterion offered the poorer clustering results on our data while the other two methods were comparable in performance. However, only the Germini method enabled us to determine an automatic cut selection method of the HC dendrogram which identifies the expected HS (clusters). Furthermore, Germini generates information regarding the topology of the staining pattern. These features might be of a particular interest for further analysis and characterization, after the detection of the hot-spots.

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O 06

Prevalence of metaplasia in salivary gland pleomorphic adenoma

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Introduction:

Pleomorphic adenoma is the most common benign salivary gland tumour. It can occur in any salivary gland, but is most frequently found in the parotid gland. Chondroid metaplasia is a frequent finding in pleomorphic adenoma. Other forms of metaplasia have been described, but are less frequently encountered.

Aim:

The aim of this study was to establish the prevalence of metaplasia in pleomorphic adenoma in our hospital population.

Material and methods:

We retrospectively reviewed all cases of salivary gland pleomorphic adenoma over a period of 5 years. We retrieved 83 head and neck pleomorphic adenoma from our archives. Results. Pleomorphic adenoma predominantly involved the parotid gland (79.5%), followed by the submandibular gland (10.8%), minor salivary glands (3.6%) and parapharyngeal space (2.4%). In a few cases, location was not available (3.6%). Four cases of metaplasia were found in our study group, three were located in the parotid gland and one in the submandibular gland. There were two cases of osseous metaplasia, one squamous cell metaplasia and one with schwannoma-like features. One case with osseous metaplasia masqueraded as a lithiasis on CT scan and showed diffuse ossification throughout an otherwise classical pleomorphic adenoma.

Conclusion:

The overall prevalence of pleomorphic adenoma with metaplasia in our study group was 5%.

ORAL PRESENTATIONS ABSTRACTS

O 07

hERα36, a new variant of ERα: Expression and potential biological relevance in breast cancer

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Estrogen receptor (ER) alpha (ERα), a 66 kDa protein, is expressed in 70% of breast carcinomas regulating steroid hormone response and prognosis. However, recent reports integrate molecular receptor heterogeneity and cross-talk with growth factors as mechanisms of endocrine resistance. Lower molecular weight ER isoforms may also be expressed, influencing the evolution of disease. We have previously identified low molecular forms of ERα in breast carcinomas, lymph nodes and distant metastases, strongly related to tamoxifen and estradiol binding capacity, higher histologic grade and impaired prognosis. A 36 KDa variant, hERα36, was reported recently at the cytoplasm and cell membrane, meeting criteria of extranuclear localization and rapid steroid signaling, related to tamoxifen resistance and impaired prognosis of breast cancer patients. In the present work, we have assayed hERα36 in 100 human breast cancer specimens, expressing or not the "classical" nuclear ERα and 5 breast cancer cell lines. We investigated the localization of the receptor, in relation to the response to tamoxifen and survival. Furthermore, we assayed rapid steroid signaling events, triggered by ERα36. We suggest that this isoform displays a discrete localization in a subset of ERα positive or negative tumors and cell lines. We further discuss the relation of ERα36 with breast cancer evolution and other biological factors, in view of tailored therapies, especially in carcinomas widely considered as non-responsive to endocrine regimens.

ORAL PRESENTATIONS ABSTRACTS

O 08

A taxonomy of epithelial human cancer and their metastases

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Introduction:

Microarray technology has allowed characterization of many different cancer sites and has potential to individualize therapy. However, due to technological differences and standardization issues, no study has evaluated the molecular profile of epithelial human cancer and their metastases in a large number of samples and tissues.

Materials and methods and results:

Expression profiles of 1566 primary and 178 metastases were studied by unsupervised hierarchical clustering. Clusters were correlated with clinicopathological data and subsequently investigated using gene set enrichment analysis and functional annotation tools. Large clusters corresponding to breast, gastrointestinal, ovarian and kidney primary tissues emerged. Chromophobe renal cell carcinoma clustered together with follicular differentiated thyroid carcinoma, supporting pathological descriptions of thyroid follicular carcinoma-like kidney tumors and suggesting that they represent a subtype of chromophobe carcinoma. We also found an expression signature identifying primary tumors of squamous origin in multiple tissues. Next, endometrioid tumors of ovary and endometrium clustered together, confirming their shared etiopathogenesis. In addition, clustering of colon and breast tumors correlated with clinico-pathological characteristics. Moreover, a signature was developed based on our unsupervised clustering of breast tumors and this was predictive for disease-specific survival in three independent studies. Next, the metastases from ovarian, breast, lung and vulva cluster with their tissue of origin while metastases from colon showed a bimodal distribution. A significant part clusters with tissue of origin while the remaining tumors cluster with the tissue of destination.

Conclusion:

Our molecular taxonomy of epithelial human cancer indicates surprising correlations over tissues. This may have an impact on the classification of many cancer sites and may advance pathological diagnosis. Moreover, these results based on unsupervised analysis yielded a signature predictive of clinical outcome in breast cancer. Additionally, we hypothesize that metastases from gastrointestinal origin either remember their tissue of origin or adapt to the tissue of destination.

ORAL PRESENTATIONS ABSTRACTS

O 09

Galectin-1 and -3 have synergistic effects on angiogenesis

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Angiogenesis is a key event in the progression of several malignancies. A significant portion of research on tumor angiogenesis has focused on Vascular Endothelial Growth Factor (VEGF). However, it has become increasingly apparent that tumor cells can utilize alternative proangiogenic signaling probably involving multiple mechanisms. It is critical to better understand this alternative angiogenic signaling pathways for the development of new targeted therapies. Evidence is accumulating that galectin family may be involved in these alternative angiogenic pathways. In the current study, we analyzed the effects of galectin-1 (gal-1), galectin-3 (gal-3) and a combination of gal-1 and -3 on angiogenesis. Galectin effects were evaluated on 2 endothelial cell lines (HUVEC and EA.hy926) on cell growth (MTT assay) and with an in vitro model of angiogenesis (Matrigel assay). While galectins have no effects on cell proliferation, we observed that gal-1 stimulates capillary tube formation in vitro. Moreover, while gal-3 alone has no significant effect on angiogenesis in vitro, the combination of gal-1 and gal-3 has a synergistic effect. This synergistic effect was abolished by addition of an inhibitor of the VEGF Receptor (VEGFR) tyrosine kinases VEGFR-1 and VEGFR-2. Gal-1, Gal-3 and the combination enhance VEGFR-2 phosphorylation. However, only the combination of gal-1 and -3 enhance VEGFR-1 phosphorylation, suggested that the synergistic effect is due to activation of VEGFR-1 signaling.

Conclusion:

These findings suggest that members of the galectin family could be alternative proangiogenic factors that activate the VEGFR signaling with differential activation depending of the presence of gal-1 and/or gal-3.

O 10

Secondary tumors within the large bowel: origin and frequency.

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Introduction

Secondary tumors are rare in the large bowel. Breast and lung cancers, as well as melanoma, are the most frequent associated primary lesions .We aim to define the frequency, origin and natural history of colorectal secondary lesions in our single tertiary referral center.

Material and Methods

All the colorectal secondary lesions from the archives of our Pathology Department over a period of 10 years and patient's chart reviewed for clinical data.

Results

During the last decade, 1200 patients underwent colorectal surgery for malignant tumors in our institution. Forty-one cases (3.4%) were secondary lesions. Thirty-three cases (80,5%) corresponded to direct colon or rectal invasion from neighboring tumors and/or by contiguous spread of pelvic metastases from other sites (ovary:13, prostate:6, urinary bladder:6, malignant melanoma:2, uterus:3, pancreas:1, sarcoma:1, anal canal:1). Only 8 cases (0.67%) were considered as hematogenous colorectal metastases. There were 6 women and 2 men (mean age: 58 years). Metastases originated from breast ductal (3) and lobular (1) carcinomas, cutaneous malignant melanoma (1), lung adenocarcinoma (1), oesophageal adenocarcinoma (1), and renal cell carcinoma (1). Five were located in the colon, one at the recto-sigmoid junction and two in the rectum. Three cases were poorly differentiated carcinomas requiring further immunostaining to confirm the primary origin. In the 5 other cases, the histological features alone were indicative of the primary site. The mean disease free interval was 7 years (range: 0 – 16 years). Three patients died from their metastatic disease (mean survival after colorectal metastasis identification: 16 months, range: 1-28 months). Two patients are alive, and 3 patients are lost to follow-up.

Conclusions

Although the frequency of secondary lesions within the large bowel is very low, this possibility should be kept in mind when confronted with a poorly differentiated or atypical tumor. Breast is the most frequent primary site. Clinical history is of help as well as immunohistochemistry.

ORAL PRESENTATIONS ABSTRACTS

O 11

CA9 expression in multilocular cystic renal cell carcinoma

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Kidney cystic disorders include developmental, acquired, benign or malignant neoplastic entities. The radiological Bosniak classification of renal cysts represents one step for the management of cystic renal masses. Bosniak cysts III and IV are suspect of malignancy and need a surgical resection. Multilocular cystic renal cell carcinoma (RCC) is a rare entity occurring in 4% of cases. Here we report the usefulness of carbonic anhydrase 9 (CA9) antibody as a marker of RCC in multilocular cystic lesion of the kidney.

We present 2 cases of multilocular cystic mass of the kidney classified on CT-scan as Bosniak III in a 55 year old man and Bosniak IV in a 68 year old woman. A nephrectomy was performed in both patients. The lesions were grossly similar and measured respectively 4,5 and 5,2 cm. A unique multilocular cyst with thin septa was observed without solid area. Microscopically these septa were fibrous and covered by a single layer of cubic or flat clear cells. There was no nuclear atypia. Some nests of clear cells were focally seen in the septa of the first case. Immunohistochemical staining for cytokeratin was positive in the clear cells in both cases and CA9 was only positive in the first case diagnosed as a multilocular cystic RCC. Second case was diagnosed as a simple renal cyst.

The radiological Bosniak classification helps for the follow up of renal cystic lesion but is not diagnostic. CA9 is not expressed in normal kidney and is positive in 98% of RCC. Here we show the utility of this marker for multilocular cystic RCC diagnosis. Further study on larger cohort, biopsy or cystic fluid is needed to confirm this preliminary result. CA9 antibody could improve the diagnostic of multilocular RCC in the context of renal cyst.

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POSTERS

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POSTERS ABSTRACTS

P01

Clear cell sarcoma of soft tissue arising in unusual sites: report of 3 cases.

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INTRODUCTION:

Clear cell sarcoma (CCS) is a rare neoplasm most frequently arising in the deep soft tissue of the extremities of young patients. Histologically, CCS display a typical nested and fascicular architecture. Tumor cells are spindle shaped, with clear or granular eosinophilic cytoplasm. Nuclei are vesicular, with prominent nucleoli. Scattered wreath-like giant cells are seen in 50% of cases. Melanin can occasionally been detected. CCS and malignant melanoma (MM) share immunohistochemical profiles and ultrastructural features, but CCS is associated with a t(12;22)(q13;q12) translocation resulting in an EWSR1/ATF1 chimeric gene, or less commonly a t(2;22) translocation fusing EWSR1 and CREB1. Recently, a few cases of CCS have been reported in unusual locations such as the gastrointestinal tract, bone, kidney and the skin. We report 3 additional cases arising in the skin (2 cases) and ileum.

MATERIAL AND METHODS:

The cases were retrieved from the consultation files of the authors. A panel of immunohistochemical studies (including S-100 protein, HMB-45 and melan-A), and FISH analysis using the LSI EWSR1 dual color break apart probe (VYSIS) were performed in all 3 cases.

RESULTS:

Clinical features are summarized in the following table:

Case	Age	Sex	Site	Size
1	30	F	Ileum	5cm
2	59	F	Thigh	2.5cm
3	60	M	Foot	2.5cm

Both cutaneous cases were intradermal; they displayed the typical architectural features of CCS. The ileal case had a non-specific histological appearance. S100-protein was expressed in all cases. HMB-45 and melan-A were only expressed in case 3. EWSR1 rearrangements were demonstrated in respectively 79%, 100% and 22% of the nuclei.

CONCLUSIONS:

Rare cases of CCS arise in unusual locations such as the skin and gastrointestinal tract, where they should be distinguished with primary or metastatic MM, because of different prognosis and treatment options. FISH analysis is an extremely useful confirmatory tool in this setting.

P02

Intestinal perforation secondary to a necrotizing vasculitis: The PAN

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Introduction:

Gastro-intestinal localizations of necrotizing vasculitis are extremely rare accounting for 5% of all locations. Complications that are usually seen are represented by haemorrhage and perforation. The most frequent reported vasculitis are Wegener's vasculitis and Shurg and Strauss one. The PAN is rarely reported.

Observation:

The authors report the case of a 50-year-old patient without a past medical history who presented with peritonitis. A surgical treatment was improved and revealed an intestinal perforation. An intestinal resection was performed. Microspic findings consisted in a largely necrotizing mucosa with multiple infiltrated vessels by neutrophils. A fibrinoid necrosis was also observed around the vessels. The diagnosis of an intestinal perforation secondary to a PAN was retained.

The originality of this case is the rarity of this complication which revealed the vasculitis

POSTERS ABSTRACTS

P03

Distal tubulopathy and glomerular proteinuria : atypical association.

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Introduction :

Gitelman syndrome is an autosomal recessive disorder characterized by tubular hypomagnesemia-hypokalemia with hypocalciuria. Glomerular diseases are rarely reported in this disease.

Case report:

A 27 years old man is admitted with hypokalemia and moderate proteinuria. Hypokalemia and hypomagnesemia are unravelled at 16 years of age during fits of spasm. No growth retardation. Absence of parental familial history but the sister of the patient would also display hypokalemia. The patient mentions two episodes of nycturia, frequent thirst sensations and salt avidity. He suffers from cramps in his calves as well as from paresthesia at the face and at the hands several times a month. The patient weighs 84 kg/185 cm, is well hydrated, has a sinusoidal cardiac rythm (60/min) and a 100/75 mmHg arterial blood pressure. Hypokalemia (2.5 mmol/L) and hypomagnesemia (1.17 mmol/L) are confirmed. Plasma bicarbonate is 33 mmol/L. Renal function is normal. Urinalysis displays inappropriate kaliuria (104 mmol/24h), abundant natriuria (266 mmol/24h), very low calciuria (< 20 mg/24h) as well as proteinuria (770 mg/24h), mainly albumin. β2-microglobulinuria is negative. Urinary sediment is negative. Echography reveals normal kidney sizes and a single simpel cyst in the right kidney. The renal biopsy shows hypertrophy of the juxtaglomerular apparatus by light microscopy and the absence of immune deposits by immunofluorescence. Electron microscopy documents extensive segments of glomerular basement membrane (GBM) either with severe irregular contours characterized by clarifications and densities varying in thickness or with regular contours of relatively thin GBM (less than 300 nm). All altered GBM display quite extensive effacement of foot processes.

Conclusion:

Ultrastructural changes of the GBM found in this patient are unusual and reported for the first time in Gitelman syndrome. Their significance and potential mechanisms await further elucidation.

P04

Dedifferentiated parosteal osteosarcoma: report of two cases.

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Parosteal osteosarcoma is a rare low-grade osteosarcoma developed at the bone surface with an excellent prognosis. This tumour exhibits a relatively simple aberration pattern dominated by ring chromosome carrying amplified material from chromosome 12 (MDM2 region). About 15% of the tumours show dedifferentiation into high-grade sarcoma (dedifferentiated parosteal osteosarcoma, DPOS), which worsens significantly the prognosis.

We reported two cases of DPOS of distal femur in a 63-year-old man and of proximal humerus in a 45-year-old woman. MRI showed large, heterogeneous and infiltrating masses suggesting high-grade lesions.

The diagnoses of osteosarcoma were performed on surgical biopsies. Then the patients received neoadjuvant chemotherapy and underwent surgical excision.

Immunohistochemical analysis was performed to evaluate MDM2 protein expression and to assess the proliferation activities using the monoclonal antibody MIB-1.

Amplification of MDM2 gene and loss of heterozygosis at the p16, TP53 and Rb-locus were studied by FISH.

On histological examination two components were reported: a low-grade osteosarcoma characterized by well formed bony trabeculae embedded in hypocellular spindle cell stroma with no significant atypia coexisting with a high-grade osteosarcoma.

The response to neoadjuvant chemotherapy was bad, based on the presence of widespread areas of viable tumor cells.

MDM2 was focally expressed in spindle cells' areas. MIB-1 showed a low proliferation index in the spindle cell compound whereas it was more elevated in the typical high grade osteosarcoma.

MDM2 gene was found amplified in both cases. Tetraploidy and a completely or partially loss of heterozygosity at the p16 and Rb-locus were detected.

We observed a close correlation between the radiological biphasic aspect of the tumours and the histology. The tumours occurred in more elderly patients that usually observed for conventional osteosarcoma, which could be explained by the presence of a low grade lesion growing slowly. Genetic data are compatible with such an hypothesis.

P05

Papular Acantholytic Dyskeratosis of the vulva: case report.

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A 36-year-old woman presented with multiples white papules on both labia majora. Her mother presented the same types of lesions but less pronounced. There were no subjective symptoms. No others cutaneous lesions were found elsewhere on the body. Pathological examination of the biopsy of the papules revealed hyperkeratosis, dyskeratosis, marked acantholysis and a "dilapidated brick wall" appearance with cells floating in the lumen of the lesion. The adnexal structures were spared by acantholysis. There was also minimal inflammation in the dermis. The diagnosis of papular acantholytic dyskeratosis of the vulva was proposed. Patients with this disease present variably pruritic, multiple isolated or groups of white papules involving the labia majora or inguinal region. By definition, there is no evidence of similar lesions elsewhere on the body. Differential diagnoses of acantholytic lesions of the vulva are Hailey-Hailey disease, pemphigus vulgaris, Darier's disease and Grover's disease. Physiopathologically, it would be interesting to search in papular acantholytic dyskeratosis of the vulva mutations of gene coding for calcium pump. Indeed, these types of mutations were found in Hailey- Hailey disease and Darier's disease, two diseases with similar histopathological lesions compared to papular acantholytic dyskeratosis of the vulva (Dhitavat et al, British Journal Of Dermatology, 2004, 150, 821-828).

Conclusion:

Wwe are not certain whether papular acantholytic dyskeratosis of the vulva is a distinct entity or a variant of other acantholytic dyskeratosis disorders. Previous reports and our case show that more established criteria required for the accurate classification of acantholytic dyskeratotic dermatoses.

P06

Stratification of thyroid malignancy risk using FNA, clinical and US features

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Objective:

To evaluate the diagnostic value of fine-needle aspiration (FNA) cytology and the additive contribution brought by clinical and US features.

Method:

Cytological and histological diagnoses were compared in a series of 924 patients who underwent US-guided FNA before surgery. We additionally developed a grading system for follicular proliferation (FP) FNA diagnosis and investigated its impact on the malignancy risk as well as the additive contribution of clinical and US features by means of decision tree analysis.

Results:

Excluding FP cases (n = 395), our data demonstrated that strictly benign or malignant FNA diagnoses exhibit great concordance with benign or malignant histological diagnoses (97.8% accuracy). Our grading system applied to the 395 FP cases revealed that grades 1, 2 and 3 were associated with a 7.7%, 17.7% and 45.7% incidence of malignancy, respectively. Decision tree analysis resulted in a classification model which involves FP grade, patient age, serum thyroglobulin level, nodule size and nodule uniqueness. This model identified a subgroup of patients with grade 1 FP nodules who were older than 50 years of age and who had a higher risk of malignancy (17.9%). In addition, high serum thyroglobulin levels were associated with a very high malignancy risk (75.0%) for patients with grade 3 FP nodules. Finally, among grade 2 FP patients, unique and large nodules were associated with a high malignancy risk of 35.1%.

Conclusions:

The integration of FP grade, clinical and US features allows the stratification of patients with FP cytology according to their risk of malignancy.

POSTERS ABSTRACTS

P07

Blastomycosis: report of a case from a non endemic region.

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Introduction:

Cutaneous blastomycosis is a rare, hard to diagnose disease that arises mostly in the tropics, especially in humid areas, affecting mainly males and rural workers. It is characterized by verrucous plaques or nodules that are slow growing and attributed to infection by different pigmented (dematiaceous) fungi. Usually the infection develops after injury, being primarily located on the lower extremities.

Observation:

We report a case of extensive blastomycosis of 2 years duration arising in a healthy 54-year-old man presented with an erythematous, violaceous plaques of the right thigh and the limb. A biopsy specimen for histopathology revealed an epidermal pseudoepitheliomatous hyperplasia. An intra epidermal blister is often present containing an inflammatory infiltrate dominated by lymphocytes and neutrophils . There is also a background of big , round and thick walled cells lying free in the epidermal blister and which are PAS + confirming their blastomycosis nature.

Discussion:

If not diagnosed and treated early, blastomycosis has a chronic evolutional course. The most frequent complication is secondary bacterial infection. Widespread lesions and long-standing cases can be treated medically.

Conclusion:

Chromoblastomycosis is rare in non endemic countries but pathologists in these regions should be aware of this diagnosis.

P08

EUS-FNA of mediastinal lymph nodes : think of alternative diagnoses

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Clinical history:

A 55 years-old male patient seeks medical help for a painful left arm dating several weeks. His past history is unremarkable. On CT scan, 2 partially calcified lymphnodes were described in the superior mediastinum at the left tracheo-oesophageal angle, measuring 17x15x10 cms and 35x23x12 cms. Echo-endoscopy was performed and the paraoesophagial lymphnode was punctured.

Cytology:

Isolated cells and small cell groups were described with abundant eosinophilic cytoplasm and round or ovoid nuclei discretely polymorphous with dense, homogeneous chromatin suggestive of a neuroendocrine tumour. This hypothesis could be confirmed on a cell block by immunohistochemistry (IHC): the tumour cells expressed CK22, Synaptophysin and Chromogranin, whereas CD56 and TTF-1 were negative. Ki 67 proliferation index was around 1%. This IHC profile was not in favour of a pulmonary primary.

Clinical History:

A thyroid US showed a suspicious hypoechoic lesion of the left lobe and hypoechoic lesions of the left jugulo-carotid chain. Thyroid scintigraphy was characterized by a predominantly right-sided, bilateral goiter. Octreoscan, PET-scan and bronchoscopic examinations were negative.

Imagery was therefore in favour of a low grade or slowly progressing tumour.

Blood Investigations showed normal levels of T4, TSH, thyroglobulin, antithyroglobulin and antithyroperoxydase antibodies, whereas CEA and Thyrocalciton levels were elevated.

Cytology:

IHC performed on the cell block from the EUS-FNA after these investigations showed thyroglobulin negativity and calcitonin positivity of the tumour cells suggesting a metastasis of a medullary carcinoma of the thyroid.

Histology:

A total thyroidectomy accompanied by a total central, anterior mediastinal and left lateral lymphadenectomy was performed, confirming the proposed diagnosis on cytology. Multiple foci of neoplastic cells were found in both thyroid lobes and 9 lymphnodes out of 45 were metastatic including the punctured mediastinal one.

Conclusion:

Medullary carcinoma of the thyroid may present as metastatic disease localized in mediastinel lymphnodes.

POSTERS ABSTRACTS

P09

Primitive cerebral EBV+ diffuse large B-cell lymphoma of the elderly.

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The 2008 WHO classification of tumours of haematopoietic and lymphoid tissues has included new lymphoma entities, amongst them the EBV positive diffuse large B-cell lymphoma of the elderly (ICDO 9680/3). We described the first patient suffering from a central nervous system location of this new provisional entity.

A 69 year-old engineer was referred to a neurologist because of cognitive and memory problems. Amongst others, he was no more able to perform sudoku faster than his wife. He also forgot the names of his grandchildren. On neurological examination, the patient appeared normal except for the five words test. After 30 minutes, he only reminds 2 out of 5 words. EEG demonstrated abnormal left activities. MRI revealed the presence of 8 disseminated supra-tentorials lesions that were contrasted enhancing and surrounded by oedema. The diagnosis of metastatic process was suggested on radiological grounds. As no extra-cerebral lesion was identified, a surgical biopsy was performed. The histological examination demonstrated brain parenchyma infiltrated by a necrotizing lymphoid proliferation mainly constituted of small T cells (CD3+). Moreover, aggregates of Hodgkin-like cells were observed. These cells were positively labelled by antibodies directed against CD20, CD30, BOB1, OCT2, LMP1, and by in situ hybridization for EBER. They were negative for CD3 and CD15. The diagnosis of an EBV positive diffuse large B-cell lymphoma of the elderly was made. The patient was treated by chemotherapy regimen resulting in complete remission and dramatic improvement of his neurologic status. Six months later, he is still free of disease.

P10

Was this strange lesion actually an angiomyolipoma?

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A voluminous tumor of the right liver was discovered by ultrasound in a 27years-old male patient complaining from a right hypochondrium pain. The biopsy revealed proliferation of epithelioid cells suggesting an hepatic origin. Nuclear atypia and mitosis were numerous. The histopathological diagnosis balanced between hepatocellular carcinoma and angiomyolipoma. Hepatocyte markers were negatives as were HMB45 and c-KIT. MelanA was positive and actin expressed in few cells. The patient was transferred to Univ. Hosp. St Luc for surgery with the suspicion of angiomyolipoma and the suggestion to exclude melanoma. The material was reanalysed. We were surprised by the negativity of HMB45, which is currently positive in angiomyolipoma and we wondered whether it could have another origin than hepatic, in particular an adrenal origin, since adrenocortical carcinomas are MelanA positive and HMB45 negative. MRI demonstrated a voluminous retroperitoneal tumor from renal or pararenal origin. At surgery, a voluminous lesion had developed in the adrenal area independently from the liver. It weighted 2450gr and had numerous necrotic and haemorrhagic areas. At the HE staining, tumoral cells were either eosinophilic or clear with nuclear polymorphism and mitosis. They had a trabecular arrangement or formed large sheets of tumoral cells. Oil red staining revealed the presence of discrete fatty droplets and the absence of melanosomes. Immunohistochemistry for HMB45, melanoma cocktail and pan-hepatocyte remained negative whereas MelanA (MART1, clone A103) was markedly positive; Ki67 was positive in more than 50% of the nuclei. Neither vascular invasion nor tumoral infiltration of the adjacent organs were found.

Conclusion:

The final diagnosis was adrenocortical carcinoma on the basis of the histological aspect, location and MelanA positivity. These tumours are rare, with an incidence of 1/million/year. Its rarity translates into a paucity of experience in its diagnosis. MelanA (MART1, clone A103) is a helpful marker for the diagnosis.

POSTERS ABSTRACTS

P11

Cutaneous epithelioid angiosarcoma: a neoplasm with potential pitfalls in diagnosis

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Introduction:

Angiosarcoma (AS) is a rare neoplasm. Cutaneous AS is the most common form of AS. The epithelioid variant of the disease, however, is a rare entity. This subset can histologically mimic non-vascular neoplasms and impose serious challenges in reaching the correct diagnosis.

Observation:

We present the case of a 50-year-old patient with cutaneous epithelioid angiosarcoma (EAS); the clinical diagnosis didn't include a vascular lesion. He had no previous radiation. The histopathological examination of the biopsy specimen by hematoxylin and eosin method was not suggestive of a malignant vascular neoplasm initially and the differential diagnoses included carcinoma, malignant melanoma and atypical lymphoid infiltrate. Only after performing immunohistochemical studies that included vascular markers, a definitive diagnosis was possible. Some cases showed unusual histopathological features.

Conclusion:

Cutaneous EAS is a rare variant of cutaneous AS that can mimic a variety of more common, non-vascular neoplasms, creating a major pitfall in the diagnosis. A careful and thorough histopathological examination and a high index of suspicion, along with appropriate immunohistochemical evaluation, can help reach a correct diagnosis and provide optimal patient care.

P12

Cutaneous large B-cell -type lymphoma: A 3- case report and review of the literature

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Background:

Primary cutaneous B-cell lymphomas form a heterogeneous group of lymphoid proliferations found on the skin. We report 3 cases of primary leg-type cutaneous large B-cell lymphoma occurring on the site a previous leg burn in one case, on the scalp in the second case and on the neck in the third one.

Material and methods:

The median age of our patients was 45 years. One patient had a history of a burn to the left leg 17 years ago. The three patients presented ulcerative lesions. Histological examination of the skin biopsy revealed the existence in the skin ulcers of atypical large lymphoid cells having an immunoblastic or centroblastic morphology and shown by immunohistochemistry to be of the B-cell phenotype, thereby evoking a diagnosis of large B-cell lymphoma. The lymphoma cells were positive for BCL2, and more weakly for BCL6, but negative for CD10. Temporary initial regression was achieved by polychemotherapy comprising cyclophosphamide, vincristine and prednisone in combination with rituximab in the three cases.

Conclusion:

This serie describes a rare cutaneous neoplasm represented by large B cell lymphoma. Venous insufficiency and lymphatic stasis have already been incriminated in the genesis of this type of lymphoma; the prior injury and resulting immune dysregulation at the burn site may have also contributed to the development of this neoplasia in one patient.

POSTERS ABSTRACTS

P13

Granulomatous gastritis: A report of 11 cases and discussion of the different cause

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Introduction: Granulomatous gastritis (GG) is a rare disease characterised by the presence of granulomas within the gastric mucosa or submucosa. Common causes of GG are Crohn's disease, disseminated sarcoidosis, infections (tuberculosis, syphilis, fungal), foreign bodies, underlying malignancy or vasculitis. Gastric TB should always be considered when dealing with granulomas in endemic areas. Moreover, gastric CD is rare in developing countries but can present as TB. The diagnosis of idiopathic GG is made only after the exclusion of other organic causes

Material and methods: We report 11 cases of granulomatous gastritis diagnosed over a 20- year-period (form 1999 - 2010).

Results: The mean age of our patients was 40 years (average 30 to 60 years). There were 9 men and 2 women. The correlation between clinical, radiological and pathological records revealed tuberculosis in 6 cases, Crohn's disease in 4 cases and sarcoidosis in 1 case.

Conclusion: GG is a very rare clinical entity, which is difficult to diagnose and it can be a serious challenge for the treating physician. It is one of the rare diseases which can have unusual presentations. Prognosis depends upon the causes which are myriad. The literature on this topic is limited, without long-term follow-up studies. Treatment should be carefully individualised with close follow-up. The possibility of gastric TB should always be kept in mind, especially when dealing with patients in endemic areas.

P14

Malignant proliferating trichilemmal cyst: a case report with review of literature.

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Introduction:

Proliferating trichilemmal cyst is a rapidly growing large cutaneous adnexal neoplasm occurring on the head and neck region of elderly women. Malignant transformation has rarely been reported in these lesions.

Observation:

We describe here the case of a 50-year-old woman who presented with a large ulcerated growth over the scalp for one year duration. Incisional biopsy revealed proliferating trichilemmal cyst with malignant transformation. She underwent wide local excision of this growth.

Conclusion:

Because of limited number of cases reported in literature, management of malignant proliferating trichilemmal cyst is controversial. Treatment mainly entails wide local surgical excision. Many other adjuvant modalities have been tried. This paper presents the diagnosis and management of one case of malignant proliferating trichilemmal cyst followed by review of the literature.

POSTERS ABSTRACTS

P15

Primary testicular large B cell lymphoma (A report of 5 cases and review of the literature)

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Background:

Testicular lymphoma was first reported by Malassez and Curling in 1866. Primary testicular lymphoma constitutes only 1-7% of all testicular neoplasms and less than 1% of all non Hodgkin lymphoma.

Aim:

The authors report five cases of primary testicular lymphoma diagnosed over a 10- year-period and highlight its diagnostic and therapeutic challenge.

Observation:

We report 5 cases of primary testicular lymphoma with a mean age of 40 years (average from 30 to 70 years). It was revealed in all cases by a painful testicular swelling. Radiological findings consisted in multiple hypoechoic masses in one case and a unique mass in the other cases. These masses corresponded in histologic examination to a diffuse intratubular lymphomatous infiltration situated away from the spermatic cord, the epididymis, ductuli efferentes and rete testis. Immunohistochemical study showed positivity for leukocytic common antigen (CD45), B-cell marker (CD20) and bcl 6. The patients underwent full staging for lymphoma showing no evidence of extra-testicular involvement by lymphoma and no lymph nodes in all cases. The diagnosis of stage I primary testicular large B cell lymphoma of germinal center-B-cell like group was made in all cases.

Conclusion:

Primary testicular lymphoma is a rare tumour whose diagnosis is based on histologic findings. There are non consensual etiological or predisposing factors. Treatment modalities consist in surgical excision, chemotherapy and radiation therapy but the accurate procedures aren't standardized. Factors that have been linked to more favorable outcomes include younger patient age, localized disease, presence of sclerosis at pathologic analysis, smaller tumour size, lower histologic tumour grade and lack of epididymal or spermatic cord involvement.

P16

Collision tumor: syringocystadenoma papilliferum with verrucous carcinoma

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A man of 45 year-old has presented a unique peri-anal, exophytic and verrucous-like lesion, measuring 21 millimeters. He did not have any medical history.

At microscopy, we have observed two tumorous pattern. The first part, superficial, was composed of acanthosic, papillomatous and hyperkeratosic epithelium.Few koïlocytic cells and basal atypia were seen without evidence of HPV infection. The second part, in the deep dermis, was contigous with the verrucous carcinoma and formed of cystic cavity lined by papillae. The epithelium was characterized by a double cellular layer: a basal myoepithelial layer and a superficial columnar layer with decapitation secretion. Stroma was composed of conjunctivo-vascular core infiltrated by plasma cells. At junction of the tumors, multilayered cells and cytonuclear atypia were seen, along with multilayer mitoses. There was no evidence of disruption of the basement membrane.

In conclusion, this is a syringocystadenoma papilliferum (SCAP) with focal syringocystadenocarcinoma papilliferum (SCACP) *in situ*, associated with a verrucous carcinoma

SCAP is rare and classified in the apocrine group. It is commonly unique, present or developed in childhood and mainly found on the scalp especially in association with a pre-existing nevus sebaceous of Jadassohn. It could occur on the face, neck, trunk and lower limbs, and rarely on eyelid, breast, arm and genitalia. Clinically, SCAP is usually a gray or dark-brown papillary or warty, crusted or exudated, exophytic lesion. Two cases in conjunction with verrucous carcinoma have been described, one located on thigh and second one on sacral region. Post-operative course was uneventful without evidence of recurrence or metastatic dissemination. SCACP is an extremely rare malignant tumor. Three cases of SCACP *in situ* were described arising from SCAP on scalp and perianal skin.

Differential diagnosis of SCAP and SCACP *in situ* should be made with hidradenoma papilliferum, a rare and benign tumor.

POSTERS ABSTRACTS

P17

Sweet's syndrome

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Sweet's syndrome, or acute neutrophilic dermatosis, is an unusual dermatologic disorder. its pathophysiology is still largely unknown and different subgroups can be defined. Clinically, it is characterized by the abrupt onset of tender or painful plaques accompanied by fever, general malaise, and neutrophilic leukocytosis.

Sweet's syndrome is a marker of several internal conditions, especially infections, inflammatory bowel disease, autoimmune disorders, and malignant neoplasm (predominantly of hematological origin).

Sweet's syndrome reflects a neutrophilic reaction that affects the skin and sometimes internal organs. For this reason it is important to reach a correct diagnosis and assess appropriately both the triggering factors and the concomitant internal involvement. There are several therapeutic approaches that achieve either the resolution or the satisfactory management of this condition.

We include here 20 patients with Sweet's syndrome and review its epidemiology, clinical spectrum, histologic features, laboratory results, differential diagnosis, pathogenic mechanisms, associated diseases, and treatment.

P18

The role of cutaneous direct immunofluorescence in the diagnosis of autoimmune bullous dermatoses

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Bullous dermatoses are a variety of autoimmune skin diseases that are characterized by the presence of bullae or blisters.

Their classification is based on the location of the blister: intraepidermal and subepidermal. The review covers the management of main autoimmune bullous dermatoses, including bullous pemphigoid and pemphigus vulgaris, linear IgA dermatosis, dermatitis herpetiformis, and bullous systemic lupus erythematosus. Patients produce autoantibodies against self-specific structures of the skin detectable by immunofluorescence techniques, immunoblotting and ELISA.

Our objective was to assess the value of direct immunofluorescence in the diagnosis autoimmune bullous dermatoses.

We included here 40 cases of different blistering lesions. Other than routine Hematoxylin and Eosin stain, direct immunofluorescence test was done in all cases. The most frequent diagnoses were pemphigus (n = 20), bullous pemphigoid (n = 11) and linear IgA dermatosis (n = 9). Clinical findings and histological examination were sufficient for the diagnosis of most cases. Direct immunofluorescence study is essential in many cases.

POSTERS ABSTRACTS

P19

Dysplastic nevi

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Dysplastic nevi have become an increasing focus clinically, with evidence that they are associated with a higher risk of developing melanoma. Since dysplastic nevi were described originally in 1978, a great deal of research has examined the epidemiology of these lesions and the genetic factors related to the development of dysplastic nevi. However, there is disagreement regarding the clinical management of dysplastic nevi and its histologic definition. We present the case of a 20-year-old man with histologically proven sporadic conjunctival dysplasic nevus and we discuss its clinical spectrum and histologic features

P20

Acrokeratosis verruciformis of Hopf. A case report

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Acrokeratosis verruciformis of Hopf is an autosomal dominant genodermatosis usually presenting with multiple planar wart-like lesions, typically observed on the dorsum of the hands and feet. The disease is very rare and the pathogenesis remains unknown. Although histology of acrokeratosis verruciformis lesions shows no evidence of dyskeratosis, a possible relationship with Darier's disease has long been postulated on the basis of clinical similarity.

We describe the case of a 20-year-old man seen in our clinic with skin-coloured, flat, warty papules localized to the dorsum of the hands and feet. Both clinical and histological findings were compatible with acrokeratosis verruciformis. We also review the disease, particularly its relation with Darier Disease.

POSTERS ABSTRACTS

P21

Lethal ricin intoxication in dogs: Toxicology and histopathology

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Introduction:

Ricinus communis (i.e. castor bean, Wonder tree) is an ornamental plant containing ricin. Ricin is a protein toxin and one the most potent plant toxins known. It may cause hypotension, gastroenteritis, depression, and death. The residual plant material from the oil extraction (used for several purposes), castor bean cake, is largely used as fertilizer. Thus intoxication can occur indirectly through ingestion of this product.

Materials and Methods:

A golden retriever male of 10 years and an Australian shepherd male of 6 years were brought in for autopsy with a suspicion of poisoning with ricine. The dogs died within 24 and 48 days after presenting symptoms of sudden prominent uncontrollable vomiting followed by abundant hemorrhagic diarrhea ending in shock and this in spite of intense therapy. Autopsy was performed and samples of gastric and intestinal content as well as of liver and kidneys were taken for further toxicological examination. For histopathology, myocard, lymphnodes, spleen, intestines, liver and kidneys were taken.

Results:

Histopathological examination of the kidneys revealed tubular degeneration and necrosis, and membranous glomerulonephritis. Additionally, myocardial degeneration with localized inflammation, lymphoid necrosis and depletion in the spleen and the mesenteric lymph nodes, and hemorrhagic ulcerative gastroenteritis were found.

Conclusion:

The current study presents the histopathological lesions of the kidney associated with lethal ricin toxicosis in dogs. Given that these animals had vomiting and hemorrhagic diarrhea, some of these histological changes could be attributed to shock as described in human beings. Additionally, the present study also presents the concentrations of ricinine, a biomarker for ricin, in various tissues. The information concerning histologic lesions and concentrations of ricinine could help in determining the lethal dose of this compound in dogs, which is currently unclear.

(Article "in press" in J Vet Diagn Invest, Vol. 22, Number 3, May 2010)

P22

Gliomatosis Cerebri, an unusual cause of late fetal immobilism.

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Neuromuscular disorders are the most frequent cause of late pregnancy fetal immobilism. We reported a case of gliomatosis cerebri as causing such disorder. Meanwhile, congenital tumors are rare, astrocytomas and teratomas appear to be the most frequent fetal neoplasms.

A caesarean was performed at term in a twenty nine year old woman because of non-reassuring fetal monitoring and breech presentation. At birth, the baby benefited from assisted ventilation because of pulmonary distress. She also suffered from major hypotonia. MRI revealed thickening of the cervical spinal cord. The lesion appeared T2 hyper-intense without contrast enhancement. In addition, a centrally located non-contrasted enhancing lesion was observed in the lower part of the spinal cord. A tumoral process was suggested. The baby died at day 11, and a post-mortem examination was performed. Cerebral examination did not reveal any supra-tentorial macroscopic lesion. The pons appeared enlarged and surrounded by muccoïd material. Spinal cord leptomeninges were white and thickened. Cross-section of the thoracic spinal cord demonstrated a centrally located yellowish parenchyma. Histological examination revealed the presence of a diffusely infiltrating tumoral astrocytes in both hemispheres, brain stem, cerebellum, spinal cord and spinal nerves. The diagnosis of fetal gliomatosis cerebri was therefore assessed.

P23

Synovial sarcoma metastasis in a Whipple resection specimen

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In January 2010 a 28-year-old woman was admitted at the Erasme University Hospital for digestive bleeding. In her medical history, we noted a synovial sarcoma of the left ankle which was treated by surgery, chemotherapy and radiotherapy in 1993. In 2008, she presented a lung metastasis, so a right middle and lower lobes resection was performed without any adjuvant therapy.

At the admission, the abdominal computed tomography showed a heterogeneous mass of 5 cm diameter in the pancreatic head, leading to a cephalic duodenopancreatectomy.

Macroscopic examination of the Whipple resection specimen displayed a smooth, soft , slightly pink mass involving the pancreatic head. The duodenal mucosa surrounding the papilla was haemorrhagic regarding to the protrusion and the ulceration caused by the mass. Microscopic analysis showed a monophasic synovial sarcoma characterized by spindle tumour cells organized in fascicles with nuclear palisading. Tumour cells had ovoid nuclei and inconspicuous nucleoli. Cytoplasm was sparse and cell borders were indistinct. Scarce mitoses were observed. No necrosis was noted. Many tumoural vascular emboli were found.

Synovial sarcoma accounts for 5 to 10% of soft tissue sarcomas; 90% occur between 15 and 35 years. Over 80% arise in deep soft tissue of extremities. The t(X,18)(p11;q11) is the cytogenetic hallmark of synovial sarcoma. Common metastatic sites are lungs, bones and also regional lymph nodes.

Metastatic disease to the pancreas represents less than 2% of all pancreatic malignancies, although isolated pancreatic metastases are rare. The effectiveness of pancreatic metastasectomy depends on the type of malignancy, the best outcome is observed for resection of metastatic renal-cell cancer. The outcome for patients with metastatic sarcoma isolated to the pancreas hasn't, however, been assessed yet.

P24

Validation of HER2 testing by FISH and IHC in gastric carcinoma using tissue microarray

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Amplification of the HER2 gene and over-expression of the HER2 protein occurs in 6 to 35% of gastric cancer, which is a characteristic associated with poor prognosis. In the first phase III study, the ToGA trial, HER2-positive patients with advanced gastroesophageal and gastric adenocarcinoma were randomized to receive 5-fluorouracil/capecitabine and cisplatin either alone or in combination with trastuzumab. A statically significant gain in overall survival was seen in the patients who received the combined treatment of trastuzumab and chemotherapy. It is expected that the encouraging results from the ToGA trial will have an immediate impact on the management of patients and that routine HER2 testing of patients with advanced gastric cancer will be initiated within a relatively short period of time. In the present study we analyzed the HER2 status by immunohistochemistry (IHC) and by fluorescence in situ hybridization (FISH) in a retrospective analysis of 43 gastric carcinomas using Tissue Microarray (TMA). IHC and FISH results were available for 40 and 33 cases respectively. IHC HER2-scoring of 0, 1+, 2+, and 3+ was of 25, 42.5, 17.5 and 15% respectively, giving an HER2-positivity rate (2+/3+) of 32.5%. Inter-observer concordance was moderate for distinguishing negative (0/1+) and positive cases (2/3+) (kappa: 0.53). FISH amplification (HER2/CEP17 ratio > 2) was observed for 7 cases (5, 1 and 1 cases with IHC scoring of 3+, 2+ and 1+ respectively), giving an HER-positivity rate of 21%. Heterogeneity in tumors was observed in IHC and FISH; 11/40 cases and 6/33 cases were characterized by at least one spot evaluated as negative and one spot evaluated as positive in IHC and FISH respectively. In conclusion, larger prospective studies are needed for validation of primary HER2-testing modality in gastric cancer.

POSTERS ABSTRACTS

P25

How extracellular matrix is involved with the IGFBP2-IGFII complex in the proliferation of glioblastoma cell line

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Aggressiveness of glioblastomas is mainly due to the rapid growth of the tumor cells and their ability to infiltrate the brain parenchyma. These tumors invade widely as single cells anywhere within the brain, showing some tendency to infiltrate along the periphery of blood vessels walls, along the subpial glial space or along white matter tracts such as the corpus callosum.

The composition of the normal brain extracellular matrix (ECM) is unique and complex. Glycolipids, glycosphingolipids, glycosaminoglycans, glycoproteins and proteoglycans are the principal elements in the brain. ECM components widely present within the ECM in others organs, including collagens, laminin and fibronectin are limited to vascular and perivascular areas in the brain.

The unique histological pattern of invasion shown by astrocytomas, together with the unique composition of the brain ECM, suggest that ECM modulates the ability of tumor cell invasion.

Insulin-like growth factor II (IGFII) positively influences astrocytoma cell growth and motility. The biological actions of the IGFs are mediated by the type I receptor (IGF-IR). Insulin-like growth factor binding proteins (IGFBP) modulate IGF bioavailability. Modulation is performed by sequestering IGFs and therefore prevents its interaction with IGF-IR. IGFBP2 is the most abundant IGFBP in the nervous system. Post-translational modification of IGFBPs such as occurs with ECM association can profoundly alter IGFBP structure/function and hence IGF actions.

The present study aims to evaluate if some components of the brain ECM modulates the stability of the IGFBP2-IGFII complex and consequentely the IGFII bioavailability.

By means of solid-phase assays using ELISA, we analysed the ability of IGFBP2 alone and complexed to IGFII to bind with ECM components.

Among the different ECM components tested (myelin basic protein (MPB), brevican, tenascin-C, vitronectin, fibronectin, and laminin), MBP had the high ability to bind IGFBP2 alone. The affinity of IGFBP2 for ECM components is enhanced when IGFBP2 is complexed to IGFII. This is observed for MBP, fibronectin and laminin

In conclusion, some ECM components (MBP, fibronectin and laminin) can bind IGFBP2, especially when it is complexed to IGFII. These interactions probably lead to an increase of free IGFII and therefore could be enhance cell growth and motility. This phenomenon could partly explain the particular behavior of astrocytomas. To validate this hypothesis, we will now performing MTT and video microscopy cell motility assays.

NOTES

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