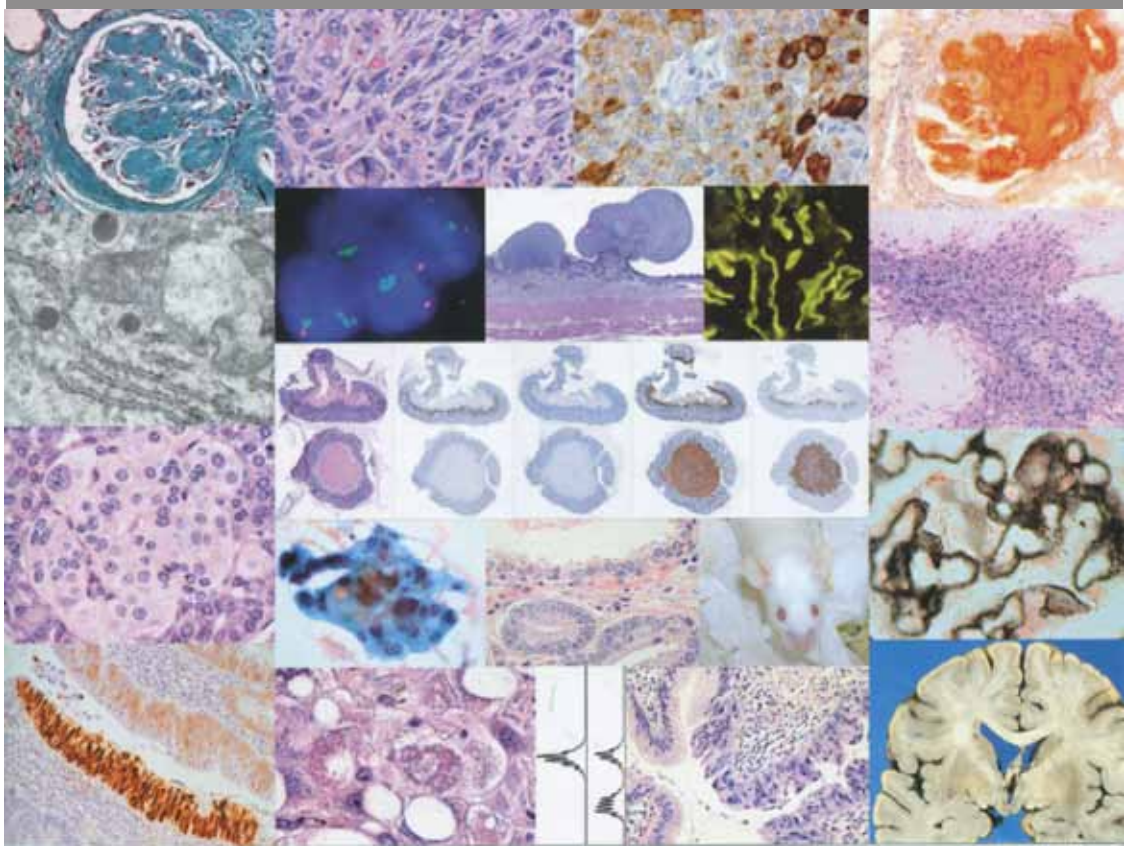
3rd BELGIAN WEEK OF PATHOLOGY 2012

April 18-21, 2012
Het Pand – Ghent



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WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

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WELCOME

Dear Colleagues,

The first two editions of the Belgian Week of Pathology (BWP) were a huge success. The previous version in 2010 was heavily disturbed by a volcano but even under these difficult circumstances, the level of the meeting and number of participants was very good and of international standards.

To organise the third meeting was therefore a big challenge, especially after two great meetings with two eminent and distinguished presidents!

After the success of the previous meetings and starting a real tradition, we have decided to program a third edition of the BWP on 18-21 April 2012. To keep up with the emerging tradition and because of the quality and splendour, the third BWP is again hosted in 't Pand in Ghent.

The scientific committee has been working on a magnificent programme to cover both the state of the art in scientific knowledge and to address other challenges which will influence our profession in the next years. We tried to reach further integration among the participating societies, creating a program that will appeal to a large audience.

We would like to thank our partners from the industry on their renewed support! Some made interesting suggestions that we worked on together.

Lastly, but most importantly, we are delighted to welcome you in Ghent for the BWP 2012!

Sincerely yours,

John-Paul Bogers
BWP 2012 President

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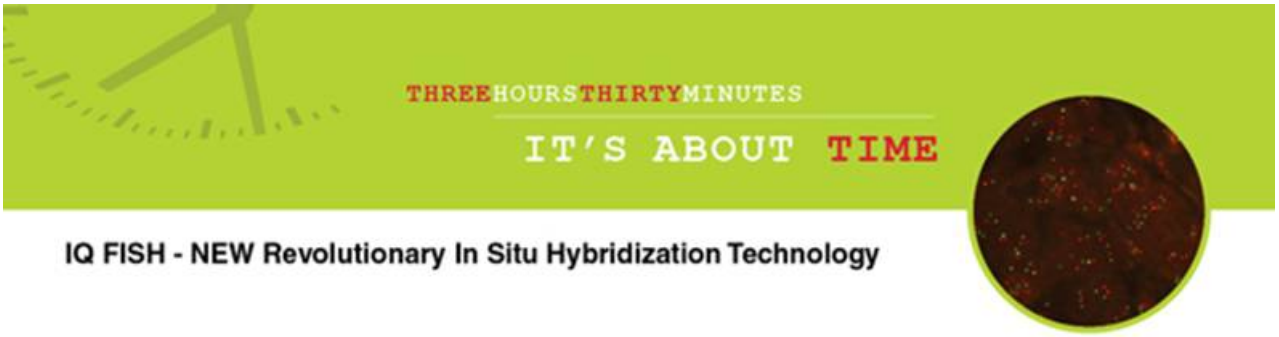

FROM CERTAINTY COMES TRUST

INDEX




GENERAL INFORMATION

Accreditation

Accreditation has been requested for ethics and economy.
From this year on, submission is done on the computers available in the exhibition area. Submission is requested once a day. You will receive a confirmation e-mail after ending the procedure.

Language

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

Abstracts

Authors were invited to send abstracts until February 27, 2012. 29 abstracts were submitted to the Selection Committee: 10 were selected as oral presentations and will be presented on Wednesday afternoon during the Free Paper Session; 19 Posters will be presented during the Poster Session on Wednesday from 13:15 to 14:00.

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

- The Boël Foundation will award the Best Oral Presentation with 2.000€
- The BWP will award the best Posters with 500€:

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 :	J.P. Bogers
Vice-President :	M. Remmelink
Past President :	I. Salmon
Treasurer :	A. Hoorens
Local Organiser :	C. Cuvelier
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Applications

- Remote pathology and consultation networks
- Integration into laboratory information system
- Research tool for tissue microarray or tissue analysis

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Vynckier A.	Beerse
Weynand B.	Mont-Godinne
Willocx F.	Brussels

WEDNESDAY
THURSDAY
FRIDAY
SATURDAY

WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

PROGRAM OVERVIEW

		REFTER ROOM Ground floor	RECTOR VERMEYLEN First floor	INFIRMARY Second floor	RECTOR BLANQUAERT Third floor
WEDNESDAY April 18	08:30-08:35	Exhibition Area Posters	Opening : J.P. Bogers		
	08:35-10:00	Exhibition Area Posters	Postgraduate Course : Soft tissues toumors		
	10:00-10:30	Coffee Break «Exhibition Area»			
	10:30-11:30	Exhibition Area Posters	Postgraduate Course : Soft tissues toumors		
	11:30-12:30	Exhibition Area Posters	Opening Lecture : P. Furness		
	12:30-14:00	LUNCH «Exhibition Area»			
	13:15-14:00	Poster Session			
	13:15-14:00	Exhibition Area Posters			
	14:00-16:00	Exhibition Area Posters	Free Paper Session		Ethics an Economy : Biobanking
	15:30-16:00	Coffee Break «Exhibition Area»			
	16:00-16:30	Coffee Break «Exhibition Area»			Ethics an Economy : Biobanking
	16:30-18:00	Exhibition Area Posters			
THURSDAY April 19					
	9:00–10:30		Oncology and molecular biology of the lower digestive tract		Molecular classification of gliomas
	10:30–11:00	Coffee Break «Exhibition Area»			
	11:00–12:00		Oncology and molecular biology of the lower digestive tract		Molecular classification of gliomas
	12:00-12:30		Memorial lecture in honour of Prof J.Hoat		
	12:30-14:00	LUNCH «Exhibition Area»		Satellite Symposium ROCHE	
	14:00-15:30		Digestive Pathology		Masterclass thyroid pathology
	15:30–16:00	Coffee Break «Exhibition Area»			
	16:00–17:30		Digestive Pathology		Masterclass thyroid pathology
FRIADAY APRIL 20					
	8:30–10:00		Masterclass laboratory accreditation		Alcohol and drug related pathology
	10:00–10:30	Coffee Break «Exhibition Area»			
	10:30–11:30		Masterclass laboratory accreditation		Alcohol and drug related pathology
	11:30–12:30		Keynote Lecture J.Krane		
	12:30–13:15	LUNCH «Exhibition Area»		Satellite Symposium QIAGEN	
	13:15–14:00			Satellite Symposium HOLOGIC	
	14:00–16:00				Alcohol and drug related pathology
	16:00-16:30	Coffee Break «Exhibition Area»			
	16:30–18:30		Masterclass pancreatic cytology		Dermatopathology
SATURDAY April 21					
	9:00-10:30		Masterclass EBUS		Cervical cancer screening anno 2012
	10:30-11:00	Coffee Break «Exhibition Area»			
	11:00–13:00		Masterclass EBUS Histopathology and cytology of the urinary tract		Cervical cancer screening anno 2012
	13:00–13:15		BWP 2012 Award : • Best Oral presentation : Boël Prize • Best Poster CLOSING: J.P. Bogers		

Every door opened could be a discovery made.

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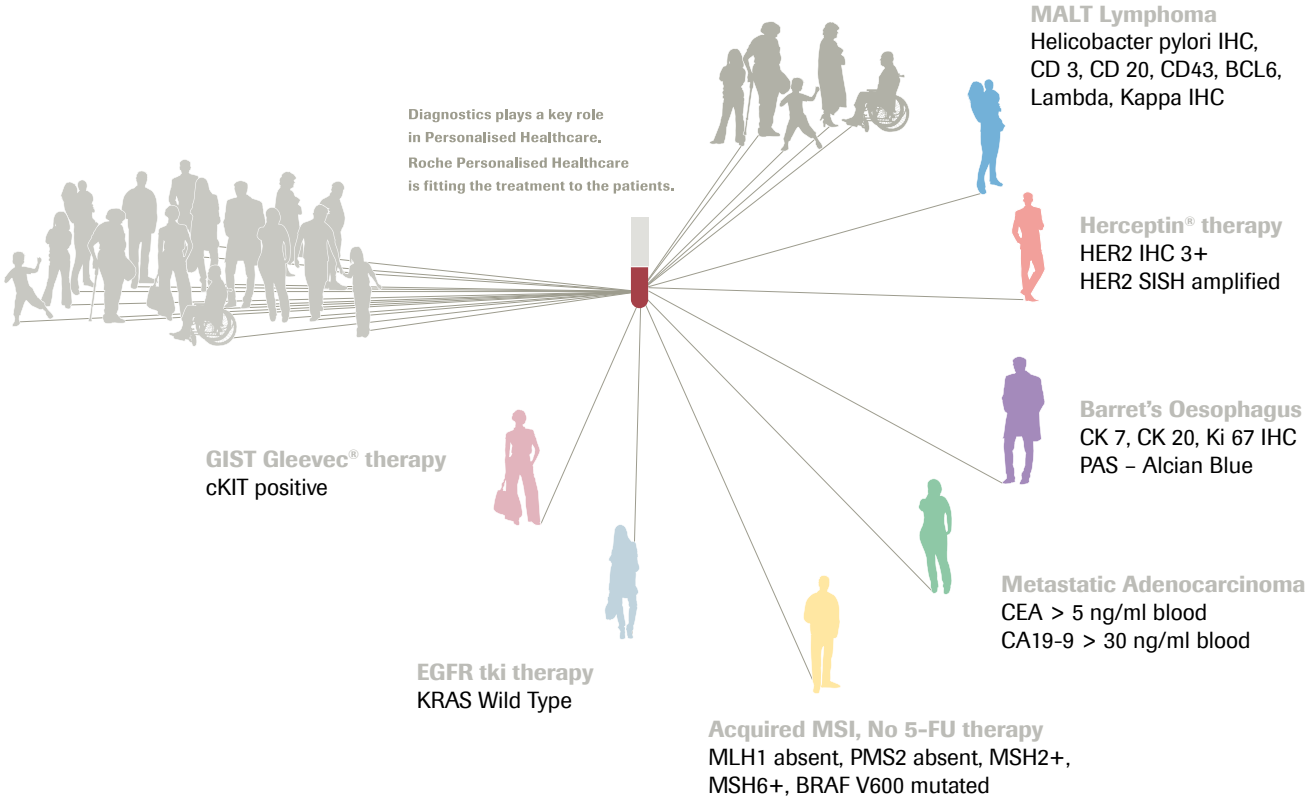

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Key tests: KRAS, BRAF and EGFR gene mutations

R.E.: Rob Geraerdts - 01TD/01-2012/03

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WEDNESDAY 18 MORNING

08:00-08:30: **Satellite Symposium**

Room Rector Vermeylen

08:30-08:35: **Welcome message** *J.P. Bogers (Antwerp)*

08:35-11:30: **Postgraduate course: soft tissue tumours**
Chairpersons: *M. Remmelink (Brussels), N. de Saint Aubain (Brussels)*

L 21 *08:35-08:50* ***Introduction*** *(M. Remmelink, Brussels)*
L 22 *08:50-09:25* ***Spindle cell tumours: a practical morphological, immuno-histochemical and molecular approach.*** *(D. Creytens, Antwerp)*
L 23 *09:25-10:00* ***Myxoid tumours.*** *(N. de Saint Aubain, Brussels)*
10:00-10:30 ***Coffee break***
L 24 *10:30-11:00* ***Pleomorphic tumours.*** *(C. Galant, Brussels)*
L 25 *11:00-11:30* ***Small blue round cell tumours****. (R. Sciot, Leuven)*

11:30-12:30: **Opening lecture**
Chairperson: *Claude Cuvelier (Ghent)*

L 01 ***The future of pathology: the next…years?*** *(P. Furness, Leicester, U.K.)*

12:30-14:00 Lunch "Exhibition Area"

13:15-14:00: Poster Session - Groundfloor

WEDNESDAY 18 AFTERNOON

Room Rector Vermeylen

14:00-16:00: **Free paper session**
Chairpersons: *N. D'Haene (Brussels), G. Boulet (Antwerp)*

O 01 *14:00-14:12* ***A real-time PCR approach based on SPF10 primers for detection and typing of human papillomavirus.***
M.I. Micalessi, G.A. Boulet, A. Vorsters, K. De Wit, G.Jannes, W. Mijs, M. Ieven, P. Van Damme, J.J. Boger / Antwerp and Ghen)

O 02 *14:12-14:24* ***Colorectal cancer in young Cambodians.***
M. Hav, S. Eav, V. Ky, C. Cuvelier, S. In, R. Kong, Y. Kheang, C. Oung, P. Pattyn, D. Lem / Ghent, Phnom Penh and Siem Reap

O 03 *14:24-14:36* ***Papillary thyroid cancer (PTC): identification of a precursor lesion.***
M. Lamba Saini, B. Weynand, J. Rahier, M. Mourad, M. Hamoir, E. Marbaix / Brussels

O 04 *14:36-14:48* ***Pathologic features, HER2 protein expression, and HER2 and CEP17 copy numbers in DCIS.***
M. Van Bockstal, K. Lambein, M. Praet, H. Denys, G. Braems, A.Nuyts, V. Cocquyt, P. Pauwels, R. Van Den Broecke, L. Libbrecht / Ghent

O 05 *14:48-15:00* ***Histopathological parameters are associated with both severity and survival in alcoholic hepatitis.***
L. Verset, E. Trépo, N. Nagy, D. Degré, T. Gustot, J. Devière, C. Moreno, P. Demetter / Brussels

O 06 *15:00-15:12* ***Immunohistochemical staining of blood and lymph vessels in canine mammary tumours.***
N. Sleeckx, L. Van Brantegem, G. Van Den Eynden, E. Fransen, C. Van Ginneken / Antwerp and Ghent

O 07 *15:12-15:24* ***Significance of intraoperative consultation in intracranial meningiomas.***
G. Dumitrescu, A. Indrei, D. Ciobanu, F. Gramada, D. Turliuc, L. Eva, D. Haba, I. Poeat / Iasi, Roumania

O 08 *15:24-15:36* ***IGF-IR and IGF-IIR/Man-6-P: new putative targets in glioblastoma?***
A.L. Trépant, C. Maris, S. Sauvage, S. Rorive, C. Decaestecker, N. D'Haene I. Salmon / Brussels and Gosselies

WEDNESDAY 18 AFTERNOON

O 09 *15:36-15:48* ***Urothelial in situ carcinoma in conventional cytology.***
A. Vasilj, S. Kojic Katovic, S. Curic Juric, H. Cupic, I. Pavic / Zagreb, Croatia

O 10 *15:48-16:00* ***Diagnostic value of the UCA1 test for detection and surveillance of urothelial cancer.***
D. Milowich, M. Le Mercier, C. Maris, N. De Neve, C. Fossion, T. Roumeguere, C. Decaestecker, I. Salmon, S. Rorive / Brussels and Gosselies

Room Rector Blanquaert

14:00-17:30: **Ethics and economy: biobanking**
Chairpersons: *C. Bourgain (Brussels), E. Marbaix (Brussels)*

L 26 *14:00-14:45* ***Belgian legal regulations on bio- and tumour banking.***
(M. Martens, Brussels)
L 27 *14:45-15:05* ***The story of the Cancer Registry, part I: from cancer incidence registration to a virtual tumour bank.*** *(E. Van Eycken, Brussels)*
L 28 *15:05-15:30* ***The story of the Cancer Registry, part II: the Belgian virtual tumour bank, a tool for translational cancer research.***
(J. Van den Eynden, Brussels)
15:30-16:00 ***Coffee break***

L 29 *16:00-16:45* ***Quality assurance in biobanking****. (R. Forsyth, Ghent)*
L 02 *16:45-17:30* ***Setting up indicators in biobanking: why and how?***
(P. Hofman, Nice, France)

THURSDAY 19 MORNING

Room Rector Blanquaert

09:00-12:00: **Molecular classification of gliomas**
Chairpersons: *I. Salmon (Brussels), C. Godfraind (Brussels)*

L 30 *09:00-09:30* ***Role of EGFR in gliomas.*** *(B. Neyns, Brussels)*
L 03 *09:30-10:00* ***MGMT methylation in glioblastoma multiforme.*** *(M. Hegi, Lausanne, Switzerland)*
L 31 *10:00-10:30* ***IDH1 and IDH2 in non-cerebral tumours.*** *(C. Godfraind, Brussels)*
10:30-11:00 ***Coffee Break***
L 04 *11:00-11:30* ***Molecular classification of gliomas.*** *(D. Figarella-Branger, Marseille, France)*
L 32 *11:30-12:00* ***Simplified approach of a molecular classification of glioblastoma.*** *(M. Le Mercier, Brussels)*

Room Rector Vermeylen

09:30-12:00: **Oncology and molecular biology of the lower digestive tract.**
Chairpersons: *P. Demetter (Brussels), K. Geboes (Ghent)*

L 33 *09:30-10:00* ***Microsatellite instability in colorectal cancer: how to deal with?*** *(P. Heimann, Brussels)*
L 34 *10:00-10:30* ***KRAS, BRAF and EGFR mutations in colorectal cancer.*** *(R. Riedl, Maastricht, The Netherlands)*
10:30-11:00 ***Coffee break***
L 34 *11:00-11:30* ***Chemokines in colorectal cancer.*** *(S. Struyf, Leuven)*
L 35 *11:30-12:00* ***Targeted therapies in malignancies of the lower digestive tract.*** *(M. Van den Eynde, Brussels)*

12:00-12:30: **Memorial lecture in honour of Prof. J. Haot**
Chairperson: *C. Cuvelier (Ghent)*

L 36 ***Lymphocytic gastritis.*** *(A. Jouret-Mourin, Brussels)*

12:30-14:00 Lunch "Exhibition Area"

Room Infirmary

12:30-13:15: **Satellite Symposium ROCHE**
13:15-14:00: **Satellite Symposium ROCHE**

THURSDAY 19 AFTERNOON

Room Rector Vermeylen

14:00-17:30: **Digestive pathology**
Chairpersons: *S. Roels (Brussels), A. Jouret-Mourin (Brussels)*

L 06 *14:00-14:30* ***Zoonosis: the other point of view or what we can learn from veterinary pathologists.*** *(A. Pospischil, Zürich, Switzerland)*
L 07 *14:30-15:00* ***Helicobacter pylori and the stomach – the end of the third decade.*** *(M. Rugge, Padova, Italy)*
L 37 *15:00-15:30* ***Helicobacter suis, the other Helicobacter.*** *(B. Flahou, Ghent)*
15:30-16:00 ***Coffee break***
L 08 *16:00-16:30* ***Use of immunohistochemistry in pathology of the upper digestive tract.*** *(J.F. Fléjou, Paris, France)*
L 38 *16:30-17:00* ***P-glycoprotein in lymphoplasmacytic enteritis in dogs, a model for Crohn's disease in humans.*** *(S. Van der Heyden, Brussels)*
L 09 *17:00-17:30* ***CMV in inflammatory bowel disease and lymphogranuloma venereum Proctitis.*** *(G. Cathomas, Liestal, Switzerland)*

Room Rector Blanquaert

14:00-17:30: **Masterclass thyroid pathology**
Chairperson: *I. Salmon (Brussels), M. Praet (Ghent)*

L 10 *14:00-14:45* ***Molecular pathways of thyroid cancers.*** *(M. Santoro, Naples)*
L 11 *14:45-15:30* ***Usefulness of thyroid molecular biology in routine practice: the pros and Cons.*** *(P. Hofman, Nice)*
15:30-16:00 Coffee break
L 12 *16:00-16:45* ***Morphology and immunohistochemistry in the study of thyroid tumours.****(C. Eloy, Porto)*
L 39 *16:45-17:30* ***Limitations in thyroid fine needle aspiration biopsy.*** *(I. Salmon, Brussels)*

FRIDAY 20 MORNING

Room Rector Vermeylen

08:30-11:30: **Masterclass laboratory accreditation**
Chairpersons: *R. Croes (Dendermonde), R. Salgado (Brussels)*

08:30-09:00 ***Accreditation of French pathology laboratories: place of the AFAQAP.***
(J.P. Bellocq, Strasbourg, France)
09:00-09:30 ***Accreditation: how does it work?*** *(V. Noten, Brussels)*
09:30-10:00 ***Postanalytical phase and involvement of pathologists in ISO 15189 Accreditation.*** *(R. Salgado, Antwerp)*
10:00-10:30 ***Coffee break***
10:30-11:00 ***Accreditation: what does it involve?*** *(I. Roland, Brussels)*
11:00-11:30 ***Accreditation: the experience of the clinical biology laboratories.***
(J.C. Libeer, Brussels)

Room Rector Blanquaert

09:00-11:30: **Alcohol and drug related pathology**
Chairpersons: *L. Libbrecht (Ghent), J.P. Bogers (Antwerp)*

09:00-09:30 ***Pathology of drug-induced liver disease.*** *(L. Libbrecht, Ghent)*
09:30-10:00 ***Alcoholic liver disease: clinicopathological features and clues for differential diagnosis.*** *(C. Sempoux, Brussels)*
10:00-10:30 ***Coffee break***
10:30-11:00 ***Drug-related pathology of the gastrointestinal tract.***
(L. Ferdinande, (Ghent)
11:00-11:30 ***Drug-related renal pathology.*** *(E. Lerut, Leuven)*

Room Rector Vermeylen

11:30-12:30: **Keynote lecture**
Chairperson: *J.P. Bogers (Antwerp)*

HPV-associated squamous cell carcinoma in the head and neck region.
(J. Krane, Boston, USA)

12:30-14:00 Lunch "Exhibition Area"

Room Infirmary

12:30-13:00: Satellite Symposium QUIAGEN
13:15-14:00: Satellite Symposium HOLOGIC

FRIDAY 20 AFTERNOON

Room Rector Blanquaert

14:00-16:00: **Alcohol and drug related pathology**
Chairpersons: *S. Roels (Brussels), A. Hoorens (Brussels)*

14:00-14:30 ***Yondelis: safety profile and hepatotoxicological differences between Cynomolgus monkey and rat.*** *(A. Vynckier, Beerse)*
14:30-15:00 ***Digital pathology within toxicologic pathology.*** *(E. Barale, Beerse)*
15:00-15:30 ***Drug induced total biliary atrophy in a young dog****.* *(M. Heimann, Loverval)*
15:30-16:00 ***Drug-induced skin diseases****.* *(V. Winnepenninckx, Maastricht, The Netherlands)*

16:00-16:30: Coffee Break

16:30-18:30: **Dermatopathology**
Chairpersons: *J. André (Brussels), M. Laporte (Brussels)*

16:30-17:15 ***Cutaneous lymphomas.*** *(J. Wechsler, Créteil, France)*
17:15-17:30 ***Questions and answers***
17:30-18:30 ***Slide seminar***

Room Rector Vermeylen

16:30-18:30: **Masterclass pancreatic cytology**
Chairpersons: *N. Nagy (Charleroi), M. Heimann (Charleroi)*

16:30-17:00 ***Fine needle aspiration biopsy of the pancreas and pancreatic cystic neoplasm****. (G. Monges, Marseille, France)*
17:00-17:30 ***Update in intraductal papillary mucinous neoplasms.*** *(N. Nagy, Charleroi)*
17:30-18:30 ***Slide seminar***

SATURDAY 21 MORNING

Room Rector Vermeylen

09:00-12:00: **Masterclass EBUS**
Chairperson: *M. Remmelink (Brussels), B. Weynand (Mont-Godinne)*

09:00-09:20 ***Granulomatous diseases.*** *(M. Remmelink, Brussels)*
09:20-09:40 ***Small cell lung cancer and metastases****. (F. Dome, Liège)*
09:40-10:00 ***Non small cell lung carcinoma****. (M. Remmelink, Brussels)*
10:00-10:30 ***Coffee break***
10:30-11:00 ***EGFR mutation testing in NSCLC****. (L. Ferdinande, Ghent)*
11:00-11:30 ***Lymphomas****. (B. Weynand, Mont-Godinne)*
11:30-12:00 ***Mesothelial pathology****. (D. Creytens and M. Luijks, Antwerp)*

12:00-13:00: **Histopathology and cytology of the urinary tract**
Chairperson: *M. Praet (Ghent)*

Cytologic, histologic and molecular approach to bladder cancer.
(E. Wojcik, Maywood, USA)

Room Rector Blanquaert

09:00-13:00: **Cervical cancer screening anno 2012**
Chairpersons: *F. Willocx (Brussels), L. Thienpont (Aalst), S. Sahebali (Antwerp)*

09:00-09:30 ***Training and education of cytotechnologists in Europe****.*
(M.L. Eide, Trondheim, Norway)
09:30-10:00 ***Current trends in cervical cancer screening****. (K. Cokelaere, Ieper)*
10:00-10:30 ***Coffee break***
10:30-11:00 ***Glandular lesions of the cervix: mimics and diagnostic pitfalls****.*
(J. Krane, Boston, USA)
11:00-11:30 ***Cervical cytology and HPV: a competition?***
(S. Sahebali, Antwerp)
11:30-13:00 ***Slide seminar***

Room Rector Vermeylen

13:00-13:15: **BWP 2012 Awards:**

- **Best Oral Presentation – Boël Prize**
- **Best Poster**

Closing remarks *J.P. Bogers (Antwerp)*

12:30-14:00 Lunch "Exhibition Area"

PRIX DU FONDS YVONNE BOËL
BOËL FOUNDATION PRIZE

Guidelines for the Scientific Award

Article 1 :
An Award of 2.000€ is offered by « The Boël Foundation ».

Article 2 :
This prize will be awarded to the best oral communication given by a researcher during the « Belgian Week of Pathology ».
The topic of the presentation will need to address research in Anatomo Pathology.

Article 3 :
The candidate must have a university degree and not be over 40 years old.

Article 4 :
The prize will be awarded after evaluation of a jury under the presidency of Prof John-Paul Bogers. The members are : Professors Pieter Demetter, Anne Mourin, Claude Cuvelier, Anne Hoorens,Tania Roskams, Philippe Delvenne.

Article 5 :
All the questions risen by the selection of the candidate and the award of the prize will be answered exclusively by the « Yvonne Boël Foundation ».

Article 6 :
The « Yvonne Boël Foundation », will be allowed to publish the abstract with the prior agreement of the winner and to mention the author's information.

Article 7 :
The Winner will be obliged to mention « Winner of Yvonne Boël's Prize » in all publications regarding this research and to specify the scientific field.

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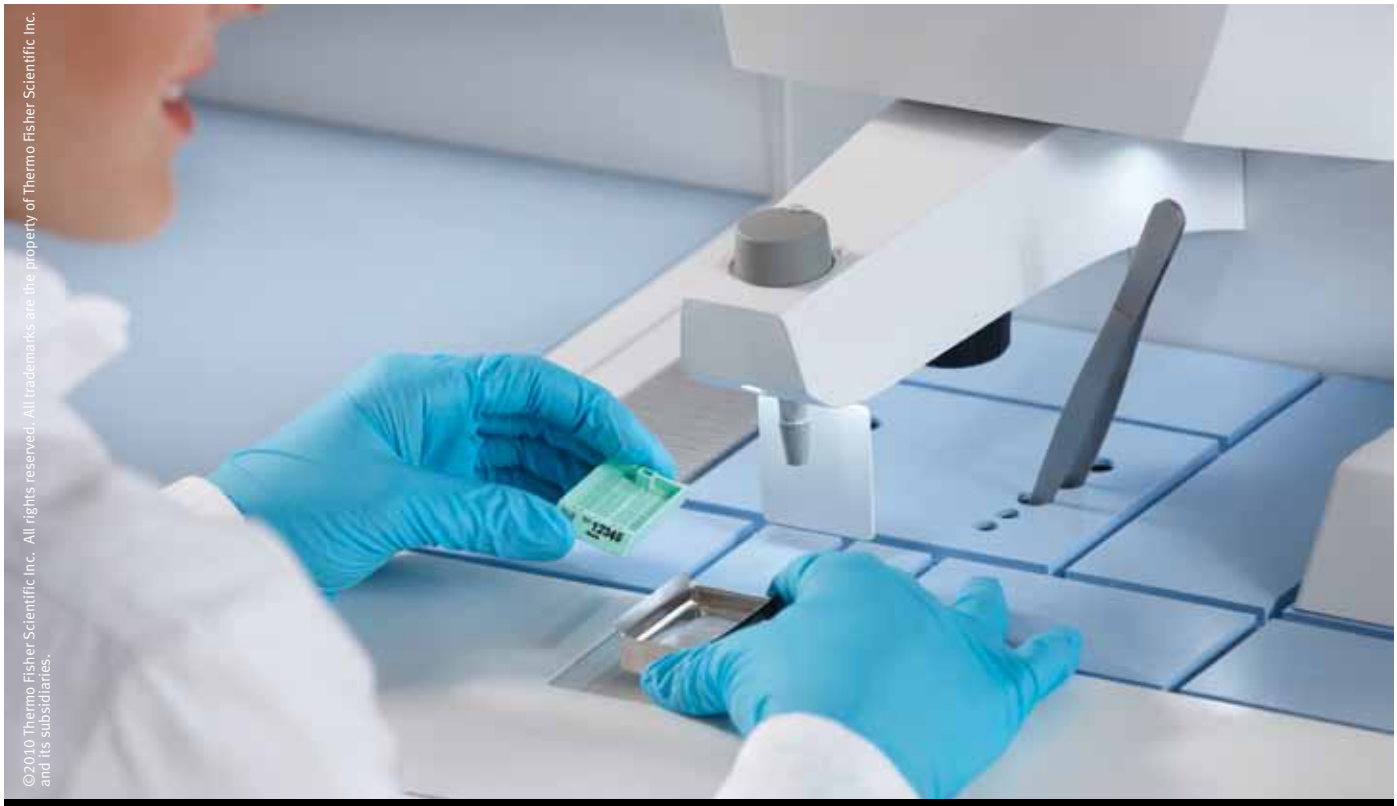

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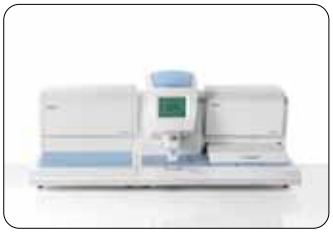

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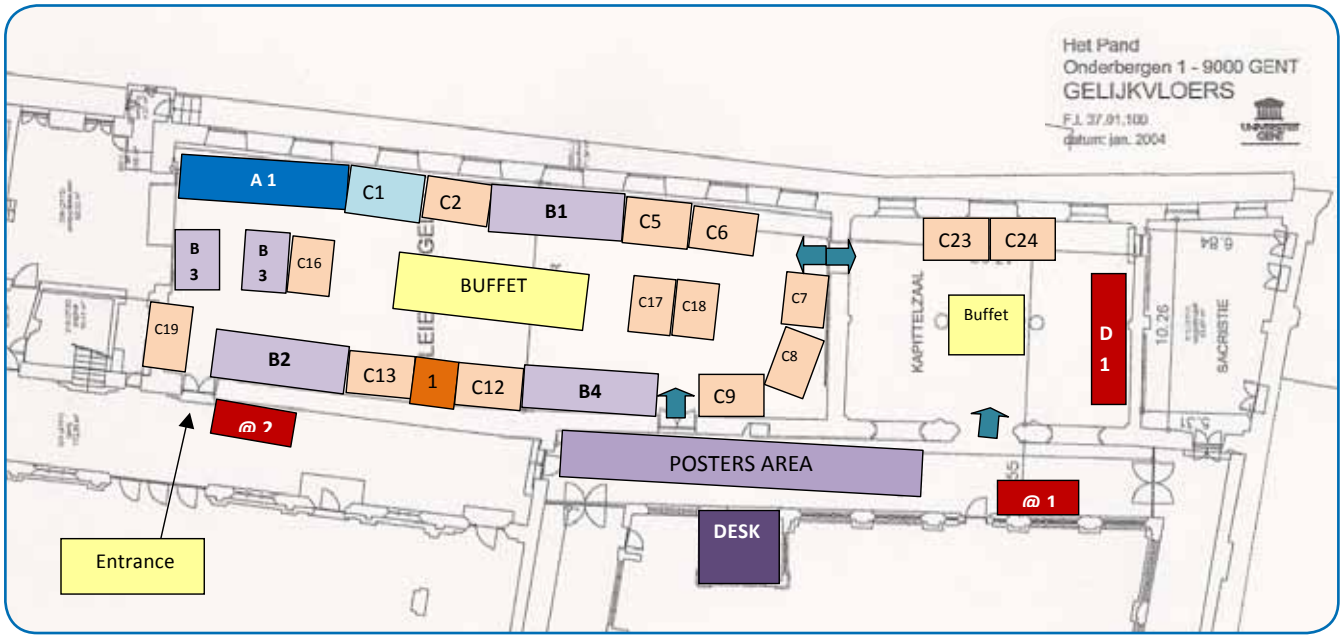

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C 24	NEOMATICS
1	BIOKE
D1	ACCO Medical

CONGRESS DINNER

Venue:

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

April 20, 2012

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

INVITED LECTURES – FOREIGN

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INVITED LECTURES – FOREIGN

L 01

The future of pathology: the next 50 years?
P. Furness, Leicester

I have been invited to speculate on how our profession will change over the next 50 years.

To address that impossible task I will first discuss in general terms what factors cause a professional group to have high status or respect within society. That apparently self-centred starting point is justified by the observation that the answer will profoundly influence the future development of the profession, in terms of the resources needed to make progress in what we do and to attract the best new recruits.

From that starting point I will consider recent trends in the way in which we do our work, and the consequences for our professional status if those trends continue. I will consider how currently foreseeable technological developments might shape how we work, what we do – and what work is done by others. And I will consider whether our current approaches to training the next generation of cellular pathologists are providing them with the best tools with which to take advantage of such changes. In passing, I will contemplate what Rudolf Virchow would probably be doing if he had been reincarnated as a trainee today.

It should be recognised that my views are based on experience in the UK, until recently as President of the Royal College of Pathologists. But I am informed that the situation in Belgium has many parallels.

Contrary to the traditional conclusion of lectures of this type, my analysis will not lead me to suggest that the future of cellular pathology is necessarily bright. Indeed, I will be delighted if someone can provide logical arguments to convince me that I am wrong. But just in case my analysis cannot be refuted, I will suggest that we need to initiate some changes.

L 02

Setting up indicators in biobanking : Why and how ?

Paul Hofman
Human biobank & Laboratory of Clinical and Experimental Pathology
University of Nice Sophia Antipolis, France

The biobanking area is highly complex, and its complexity is increasing along with its growth and demand. Due to the advancements in genetic research, stem cell research and regenerative medicine, biobanking has become ever more important and plays a key role in biomedical research. The robustness and the reproducibility of research results depend greatly on the quality and on the number of the samples used, and thus on the expertise of biobanks having supplied these samples. Undoubtedly, the recognition of a research biobank depends on the impact of the research projects conducted with samples obtained from tumour bank(s), but also on many other criteria. It thus seems important to **determine a number of indicators** within a biobank to estimate objective criteria for the performance of these structures. These indicators can allow to make some strategic decisions knowing that biobanks are expensive structures to maintain in the present hospital context. The use of these indicators could also contribute to the elaboration of an "biobank impact factor of" or so called "bioresource research impact factor" (BRIF). We describe here four major categories of indicators (quality, activity, scientific production, visibility) which seem to be useful for the evaluation of a biobank by making a proposition of allocation of coefficients for the various considered items.

L 04

Molecular classification of gliomas

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UMR 911 CRO2, team 4 : angiogenesis and tumoral microenvironment. Faculty of Medicine, 27 Bd Jean Moulin, 13005 Marseille

According to their mode of spreading two main glioma subtypes can be distinguished: circumbscribed gliomas and diffuse gliomas. Pilocytic astrocytoma (PA) belong to the first group. It is the most frequent glioma in childhood, can be cured by total surgical excision and is most often classified as grade I (WHO classification). In contrast, diffuse gliomas mainly occurred in adults and are classified into different subtypes according to their presumptive cell of origin (astrocytomas, oligodendrogliomas and mixed gliomas) and grade (II, III and IV).
Major advances have been made in the front of molecular characterization of glioma subtypes and some markers can be now used in routine practice to increase diagnostic accuracy and to predict outcome (for review Riemenschneider et al 2010).
Recent studies have shown a central role for the mitogen-activated protein kinase (MAPK) pathway in the tumorigenesis of PA. Two major alterations have been recorded in PA. The most frequent is a fusion between *KIAA1549* and *BRAF* arising from a tandem duplication within 7q34. The other is a *BRAF* missense mutation of the V600E type. *KIAA1549-BRAF* fusion mainly occur in posterior fossa PA, whereas V600E *BRAF* mutation, also encountered in pleiomorphic xanthoastrocytomas and gangliogliomas, characterized supra-tentorial PA (Schindler et al 2011).
In adut diffuse gliomas, the major advance was the discovery of *IDH* (isocitrate deshydrogenase) mutations in low grade gliomas (LGG, grade II), anaplastic gliomas (grade III) and secondary glioblastomas (grade IV, Yan et al 2009). *IDH1* mutation occurred at codon 132 and the major mutation is R132H, whereas *IDH2* mutation occurred at codon 172 and is a rare event. The anti-R132H antibody that can be used in formalin-fixed paraffin-embedded specimen constitutes an invaluable help in routine practice (Capper et al 2009). In contrast to LGG, grade III and secondary glioblastomas, primary glioblastomas do not carry *IDH* mutation. In addition, lack of *IDH* mutation (*IDH*- gliomas) has been reported in gliomatosis cerebri and in some LGG of dismal prognostic (Seitz et al 2011, Metellus et al 2010)
Before the discovery of *IDH* mutations, two recurrent molecular alterations were known in LGG: *TP53* mutations and 1p19q codeletion (as the result of t (1;19) (q10;p10) translocation, Jenkins et al 2006). *TP53* mutations (or p53 expression recorded as p53+) characterized astrocytomas whereas 1p19q codeletion (designed as 1p19q+) is a hallmark of oligodendrogliomas.
According to the three major genetic alterations recorded in adult LGG, four molecular groups can be set up: group 1 IDH+/p53-/1p19q-, group 2 IDH+/p53-

/1p19q+, group 3 IDH+/p53+/1p19q- and group 4 triple negative gliomas (Figarella-Branger et al 2011). These groups are designed because 1) 1p19q codeletion and p53 mutation are mutually exclusive (rewieved in Riemenschneider et al 2010 2) *IDH1* mutation preceeds *p53* mutation or 1p19q codeletion (Watanabe et al and 3) triple negative gliomas exist (Metellus et al 2010).
Interestingly, by using this classification we were able to classify all LGGs. From the study performed by Kim et al 2010 we also learnt that 93% of LGGs were accurately classified into these four groups, and the percentage of cases recorded in each subgroups remained in the same range. This molecular classification predicts overall survival on uni- and multivariate analysis (P=0.001 and P=0.007 respectively).
Taken together we strongly recommend to search for R132H expression in all diffuse gliomas for diagnostic and prognostic relevance because it has been reported that R132H positive gliomas have a better prognostic than their negative counterparts and thus whatever the grade (Sanson et al 2009). When negative, search for minor *IDH1* and *IDH2* mutation is recommended in grade II and III gliomas to be able to classify them as IDH+ or IDH-. In these gliomas search for p53 expression and especially 1p19q deletion is of importance to better classify them. Moreover 1p19q codeletion is predictive of treatment response. In all cases it is of importance to correlate pathological features with imaging to rule out gliomatosis cerebri or an infiltrative part of a glioblastoma. Because glioblastomas often display EGFR amplification (often associated with strong and diffuse expression, Coulibaly et al 2010) search for this genetic alteration might be of diagnostic interest but its prognostic relevance is a matter of controversy.

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L 06

What can we learn from veterinary pathology?

Andreas Pospischil

Institute for Veterinary Pathology, University of Zurich, Switzerland

The increase of world population to more than 8 billion as estimated by CDC is a huge challenge for the development of nutrition worldwide. In addition increasing wealth of a growing middle class population especially in non-OECD countries will raise the number of daily meals per person with higher consumption of milk, eggs and meat per person. Food production in consequence will be even more globalized with respect to movement of goods and people followed by a possible spread of infectious agents across the planet. Risks of this scenario are summarized and termed 5 "T" (Trade, Travel, Transport, Tourism, Terrorism) by OIE (WHO of animal health).

The frequency of infectious diseases in man dropped continuously during in the early to mid 20th century due to increased hygiene, vaccination and antibiotics, however, since the last decades of the 20th century infectious disease are on the rise again either as emerging (new diseases or infectious agents crossing species barriers) or reemerging infections. According to CDC 70% of these infections are vector-borne or zoonotic.

Together with animals and plants man lives together in one world and one environment initiating the One Health movement (http://www.onehealthinitiative.com). This initiative is a movement to forge collaborations between physicians, veterinarians, dentists, nurses and other scientific-health and environmentally related disciplines to expand interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment.

In the past a large proportion of infectious diseases in veterinary medicine has been diagnosed through veterinary pathology in cooperation with other veterinary diagnostic laboratories it is important in this context in to look into the chances and possibilities of veterinary pathologic diagnostics in cooperation with other veterinary diagnostic laboratories. In fact veterinary pathology in cooperation with other veterinary diagnostic laboratories was able to diagnose Rinderpest outbreaks that followed Tschingis Khan`s & Napoleon`s conquests in the seized countries and in addition during the Gulf war in 1990 fleeing Kurds took Rinderpest infected goats from Iraque to Eastern Turkey. From there one of the severest Rinderpest epidemic in the 20th century resulted.

The appearance of emerging and / or reemerging infections depends on the (new) host species, the infectious agent and the ecosystem. Other factors involved are bio- or agroterrorism, biological weapons, changing human demographics and behavior including human migration due to war, famine or economic disasters.

Some examples are: reduction of rainforest, global warming etc. may lead to

increased populations of feral birds spreading salmonellosis or avian influenza virus, population pressure in feral animal populations induces movement of feral animals into more human populated areas, anthrax bioterror in 2001.

In addition a number of zoonotic infections can by transmitted to humans by pets (dogs, cats, reptiles etc. like Bartonella henselae, Leishmania, Yersinia pestis, Baylisascaris procyonis, Echinococcus multilocularis.

Which actions and information can veterinary pathology in cooperation with other veterinary diagnostic laboratories offer: diagnoses of emerging & re-emerging diseases through regular investigation of diseased / dead animals by post mortem investigation in farm and pet animals species in specialized laboratories and abattoirs, information of authorities and reporting of unusual findings, communication with human medical persons, farmers and animal owners.

In Europe diagnostic procedures in veterinary diagnostic laboratories including veterinary pathology follows ISO/IEC 17025 accreditation. Internationally there is a well developed communication network through:

promed: http://www.promedmail.org

OIE: http://www.oie.int/eng/en-index.html

L 09

CMV in inflammatory bowel disease and lymphogranuloma venereum proctitis

Gieri Cathomas, Kantonales Institut für Pathologie, Liestal, Switzerland

Infections of the gastrointestinal tract are wide spread worldwide and a major cause of morbidity and mortality. However, compared to the frequency of intestinal infectious diseases, pathologists are not so commonly confronted with this diagnosis as the majority of these affections show an acute clinical course in which usually no biopsy is taken. Furthermore, despite some infections agents can be easily seen by histopathology, as Herpes- or Candida esophagitis, Helicobacter gastritis or *Giardia lamblia*, many infections lead to a characteristic but finally unspecific histological picture, especially in the lower gastrointestinal tract. In this presentation, two types of infections which may be of interest for the everyday work of pathologists, the Cytomegalovirus (CMV) infection in patients with chronic inflammatory bowel disease (IBD) and the more recently described type of characteristic proctitis especially in men who have sex with men caused by chlamydia trachomatis serovar L.

CMV in inflammatory bowel disease: CMV is a member of the *Herpesviridiae* (Human Herpes virus 4) and serolgical evidence of infection is observed in 40-100% of the general population [1]. As other herpes viruses, following primary infection, usually in childhood or adolescence, CMV persists lifelong. In individuals with appropriate immunity, this persistence of CMV has no clinical impact. In contrast, in patients with immunosuppression, especially in patients following bone marrow/stem cell or solid organ transplantation and in patients with AIDS, CMV may lead to severe disease, including prolonged fever, pneumonitis, retinitis, encephalitis and other end organ diseases. However, the presence of CMV detected by various techniques in the blood and/or tissue of patients in different clinical settings may not always be associated with CMV induced disease. Therefore, it is clinically important to distinguish CMV infection characterized by the presence of CMV in blood and/or end organs without associated symptoms and CMV disease defined by CMV infection and the presence of related clinical symptoms or end organ disease. As this distinction is often not straightforward, in clinical settings where CMV is common and may be even fatal, precise definition of CMV infection in discriminating from CMV disease have been developed, namely in the field of bone narrow and organ transplantation [2].

In the gastrointestinal tract, CMV infection may affect all levels of the organ system, from the oral cavity to the anus. Acute primary infections in the immune competent host is usually of transient nature and rarely leads to major disease in the gut, although recently it has being suggested that it may be more common than generally

anticipated [3]. It hast long been recognized that the frequency of CMV infection is increased in patient with IBD. This is especially true in patient with ulcerative colitis, whereas CMV infection is rare in patient with Crohn's disease [4]. The role of CMV in patient with IBD has raised a number of controversies about the relevance of the virus as being either an innocent bystander or complicating or aggravating the disease. Other unanswered questions include the prevalence of infection and the diagnosis of disease.

Overall, the incidence of CMV infection in IBD is low, generally considered between 3-5% [5]. However, in patient with steroid resistance, up to 36 % of CMV infection have been reported, especially in resection specimens (reviewed in [4]). On the pathologist's point of view, the diagnosis is still based primarily on the detection of characteristic CMV inclusions in biopsies or surgical specimens. Classical inclusion bodies, the well known owl eyes due to the characteristic basophilic inclusion surround by a clear halo and condensed, irregular chromatin of the nuclear membrane, are, however, not so commonly seen as they are typically present in epithelial cells which are quite rarely infected in the gut. Most commonly, CMV inclusions can be found in granulation tissue of ulcers and here especially in the endothelial cells as well in stromal cells including fibroblasts but also smooth muscle cells. Here, however, the inclusion bodies are less characteristic and may be overlooked. In addition, degenerative nuclear changes of stromal or epithelial cells or ganglion cells can be erroneously diagnosed as CMV infected cells. Generally, the specificity for H&E has been reported to be 92-100% whereas a much lower sensitivity has been described, ranging from 10-87% [4]. However, all these numbers have to been taken with caution as the evaluation of a diagnostic test needs a gold standard which is often differently defined within the various studies.

The use of immunohistochemistry can improve sensitivity and specificity. However, appropriate antibodies, namely antibodies against the immediate early antigen of CMV should be used to reach the highest level of sensitivity. Extensive ulceration have been shown to be more commonly associated with superimposed CMV disease and within the ulcers, CMV infected cell have been described especially of the granulation tissue of the base of the ulcers. Recently, the use of the polymerase chain reaction (PCR) has shown to be also effective in detection of CMV DNA in tissue. However, the mere presence of CMV DNA by PCR may be a low predictor of CMV disease and should be replaced by quantitative PCR which have shown to be more predictive for CMV associated disease [6, 7]. The relevance of CMV in causing disease may be supported by semi quantitative analysis considering the number of infected cells in a lesion and the extend of the infected tissue within the colon [8]. In addition, the presence of an increased viral load in the blood of the patient may support this diagnosis. From the clinical point of view, based on the data available today, low level and moderate CMV infection may need no or little reduction of immunosuppression in a patient with a flare up of ulcerative colitis. In a severe, extensive CMV infection in a steroid resistant patient, reduction of immunosuppression and/or antiviral therapy should be considered, especially if evidence of a generalized CMV disease are present [7, 9].

In summary, pathologists should always seek for CMV in biopsies of patient with IBD, however, the probability to detect the virus is highest in granulation tissue of long standing ulcerative colitis, especially in steroid refractory disease. In these tissue samples, immunohistochemistry should be applied and semi quantitative estimation of the extent of infection may be given. Evidence of extensive CMV infection in these patients is of predictive value, as it may lead to a reduction of immunosuppressant and/or antiviral therapy may be given.

Lymphogranuloma venereum proctitis: Chlamydia trachomatis is an obligate intracellular bacterium which encompasses serovars A to L and leading to trachoma, inclusion conjunctivitis and urogenital infections. Lymphogranuloma venerum (LGV) is a sexually transmitted disease caused by *C. trachomatis* serovar L1 – L3, causing a more invasive inflammation characteristically associated with inguinal lymhadenopathy. This disease has been described back to the early Thirties of the last century considered being an exotic disease imported from endemic area as Africa, Caribbean and Asia. At the same time, it has also been well described that this disease leads to proctocolitis with ulceration and granulomatous inflammation. Recently, however, a new epidemic of Chlamydia trachomatis proctitis has been described in Western Europe especially in men who have sex with men and are often, but not always, HIV-positive [10]. Clinically, the patients claim about mucous discharge, altered bowel habits, rectal bleeding, anal pain and tenessems [11]. Histologically, only mild distortion of crypts is seen but cryptitis and crypt abscesses are common. Granulomas, however, are only seen in less than half of the patients. The main differential diagnosis is IBD and in the case of the detection of granulomas, namely Crohn's disease [11].

Although the pathologist can not provide an affirmative diagnosis, he or she should consider LGV proctitis in the adequate clinical background and a persistent, slightly "atypical" chronic proctitis. The affirmative diagnoses can be made PCR analyses from rectal swap specimen, confirming the L-serovar [12]. In addition, PCR detection of the bacterium from paraffin embedded tissue can also be applied. Obviously, the correct diagnosis is predictive as the disease can be successfully treated by antibiotics over an extended time period [13].

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L 10

"Molecular pathways of thyroid cancers"

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In the past two decades, genetic lesions associated to thyroid cancer have been unveiled, with mutations in RET or BRAF, and PPARG or RAS associated to papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), respectively. Undifferentiated thyroid carcinoma (ATC) shows both mutations in the MAPK signaling cascade (RAS or BRAF) and in the tumor suppressor P53. Finally, medullary thyroid cancer (MTC), that arises from neuroendocrine C cells, features either mutations in RET or in RAS. This knowledge has offered solid ground for studies aimed at exploiting these molecular lesions for a molecular diagnosis of thyroid cancer in fine-needle aspirates. Moreover, some of these oncogenes code for protein kinases and this has suggested kinase targeted therapy as one possibility for patients with radio-iodine refractory follicular-cell derived thyroid cancer or for MTC. RET kinase inhibitors that function in the nM range are undergoing clinical testing in patients affected by thyroid cancer, MTC in particular. These compounds are multi-target and share the ability of inhibiting not only RET but also VEGFRs, thereby exerting both anti-tumor and anti-angiogenic activity. We have identified ZD6474, vandetanib, as a potent RET kinase inhibitor; ZD6474 showed compelling activity in a Phase III clinical study in MTC patients and has been recently approved by FDA and EMA for the treatment of locally invasive or metastatic MTC. On the other hand, BRAF kinase inhibitors are promising agents for aggressive PTC or ATC. One of them, vemurafenib, has gained FDA approval for melanoma, another human cancer that often features mutations in BRAF. One problem that could emerge with the use of kinase inhibitors is molecular resistance formation. This may be caused by activation of regulatory circuits that compensate for the blocked kinase or by mutations in the targeted kinase that abrogate drug binding. Combination therapies would be required to overcome resistance formation.

L 11

Usefulness of thyroid molecular biology in routine practice: The pros and cons

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The development of molecular biology analyses in thyroid pathology is currently active and provides new diagnostic tools with the aim of accurately distinguishing malignant and benign thyroid tumors. This is particularly useful as most of these analyses can be done preoperatively on thyroid fine-needle aspiration biopsy samples. Furthermore, molecular biomarkers may have a promising role on account of their ability to predict the prognosis of thyroid tumors. Moreover, identification of molecular markers as well as a better understanding of thyroid carcinogenesis are attractive prospects for the development of innovative targeted therapies, particularly in patients with metastatic iodo-resistant thyroid carcinoma. To date, four types of somatic genetic alterations are known to have a potential interest for the diagnosis and/or prognosis of follicular cell-derived thyroid carcinomas: *BRAF* and *RAS* mutations, and *RET/PTC* and *PAX8/PPAR*γ rearrangements. Other recent molecular biomarkers have been investigated in thyroid oncology, in particular on different microRNA signatures. The purpose of this presentation is to describe the different aspects of ancillary methods, including molecular biology, which are of current interest for the diagnosis, prognosis and/or treatment of follicular cell-derived thyroid carcinomas.

L 12

Morphology and immunohistochemistry in the study of thyroid tumours.

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The most frequent problem raised in routine examination of thyroid tumours concerns the differential diagnosis of well differentiated follicular patterned tumours. The follicular adenoma (FA), the follicular variant of papillary thyroid carcinoma (FVPTC) and the minimally invasive follicular thyroid carcinoma belong to this group of tumours and may be separated from each other with the help of two basic, but often difficult, morphological key features: nuclear features and growth pattern and invasion, namely capsular and vascular invasion.

The occurrence of papillary thyroid carcinoma (PTC) type nuclei is regarded as the most important single feature to define PTC. However, the nuclear features *per se* are not enough to define malignancy. For instance, in Hashimoto thyroiditis the nuclei can be undistinguishable from PTC nuclei occurring in follicular cells intermingled with inflammatory cells throughout the whole thyroid, without the formation of a tumour (nodule). The constitution of a more or less well defined tumour represents a condition to admit that a lesion belongs to the group of neoplastic (benign or malignant) lesions. A particular setting where such tumour formation may be not so obvious is the diffuse sclerosing variant of PTC that diffusely involves a single lobe or the whole thyroid exhibiting squamous aspects and psammoma bodies in a fibrous background. The identification and report of particular morphological variants of PTC such as the diffuse sclerosing variant, tall cell variant, columnar cell variant and microcarcinoma, provide some prognostic information that cannot be substituted by the expression of any known immunohistochemical marker.

In Hashimoto thyroiditis, like in other lesions, the growth pattern and invasion appear to be more important than the nuclear features. This observation has implications in the cytological examination setting where the nuclear features, which are observed without the perception of the architectural general organization, must be interpreted together with the ultrasound information on the growth pattern/invasiveness of the lesion.

At this point we asked ourselves: is the evaluation of growth pattern/invasion also important in follicular patterned tumours with unquestionable PTC type nuclei? The answer is YES, we think it is important. In a clinico-pathological study of a series of 75 classic PTC (CPTC) and FVPTC cases, we evaluated the relative contribution of the morphological features of the tumours for the occurrence of nodal metastases. The morphological features most closely related to the occurrence of nodal metastases were extra-thyroid extension and poorly circumscribed/infiltrative growth pattern, in both CPTC and FVPTC. Additional

features significantly associated to nodal metastases were multicentricity in the CPTC and vascular invasion in the FVPTC. These morphological findings highlight the importance of growth pattern and invasion and support the subdivision of the FVPTC.

The FVPTC encompasses the infiltrative subtype (I-FVPTC), which shares most of the features of CPTC giving rise to nodal metastases, the encapsulated subtype (E-FVPTC), which appears to be related to minimally invasive follicular carcinoma, and the multinodular/diffuse subtype which typically occurs in young women and gives rise to blood borne metastases. In our hands, none of the E-FVPTC cases presented extra-thyroid extension, lymph vessel invasion or nodal metastases, at variance with I-FVPTC and CPTC cases. In an attempt to contribute to the understanding of the differences in the behaviour of the I-FVPTC and the E-FVPTC, we evaluated the intratumoural lymph vessels density using the immunomarker D2-40 in a series of E-FVPTC, I-FVPTC, and CPTC. Only one case of E-FVPTC (8.3%) had intratumoural D2-40-stained vessels in contrast to their presence in 76.5% of the cases of I-FVPTC. Intratumoural LVD determined by D2-40 expression correlated with the occurrence of extra-thyroid extension, lymph vessel invasion and nodal metastases in PTC cases.

The reasons for the behaviour of poorly circumscribed/infiltrative PTC are probably multiple and deserve further studies. In a recent study, we found that at the periphery of poorly circumscribed, frequently *BRAF* mutated PTC there was an increased expression of TGFb associated to morphological features of invasiveness, featuring the so-called epithelial-to-mesenchymal transition, and presence of nodal metastases. The above mentioned relationship between growth pattern/invasion and *BRAF* mutation reinforce the assumption that a morphological frame is crucial for the understanding of the molecular alterations in thyroid tumours.

The evaluation of growth pattern and invasion is very important for the risk stratification of PTC. In encapsulated follicular patterned tumours with doubtful or absent of PTC type nuclear features, the evaluation of growth pattern and invasion is also crucial to establish a correct diagnosis. An encapsulated follicular tumour with evident signs of invasion and doubtful nuclear features has been designated 'well differentiated carcinoma, NOS'. Although we do not sympathize with this designation, we use it occasionally for two main reasons: some follicular patterned carcinomas of the thyroid are difficult to characterize because they have intermediate nuclei or different types of nuclei, and the treatment is similar for 'CPTC,' 'angioinvasive follicular carcinoma' and 'angioinvasive, well differentiated carcinoma, NOS'. The problem is quite different when an encapsulated follicular tumour without signs of invasion (capsular and vascular) discloses doubtful nuclear features. In this setting, the classification 'well differentiated tumour of uncertain malignant potential' can be used, although as rarely as possible. Such diagnosis should only be made in cases in which one has a thorough evaluation of the whole capsule of the tumour. The detection of several immunohistochemical and molecular markers has not proved to be diagnostically helpful in this setting. We realize that the utilization of 'uncertain malignant potential' in a pathology report creates a big problem to clinicians, not only because of its 'uncertainty' but also because it is not yet associated with any firmly established management protocol. In our institution, well differentiated tumours of uncertain malignant potential are treated

conservatively.
The results obtained so far in our retrospective study of 239 cases of well differentiated thyroid carcinomas diagnosed in the Hospital S. João and Porto Cancer Institute, from 1978 to 2003, which displayed nodal and/or blood born metastases, has shown that in this group there was not a single case of well differentiated tumour of uncertain malignant potential, nor any case of minimally invasive, non angioinvasive follicular carcinoma or encapsulated, noninvasive FVPTC (Magalhães et al, unpublished results).
Similarly, we could not also find any metastatic follicular tumour of uncertain malignant potential and we think that follicular tumours in which capsular invasion is the single sign of malignancy, should be treated conservatively (lobectomy or lobectomy plus isthmectomy).
The designation 'Follicular tumour of uncertain malignant potential' advanced by Dillwyn Williams and recommended by the WHO since 2004, applies if there are no unequivocal signs of invasiveness in an encapsulated tumour without typical PTC type nuclei. For the time being, the histological analysis of many samples of the tumour capsule is thus necessary to make the differential diagnosis in borderline cases, namely *via* the identification of vascular invasiveness. The search for such signs should be made in numerous H&E-stained sections involving the capsule of the tumour. In our hands, as stressed above, immunostaining of capsular vessels has not proved to be diagnostically useful.
The morphological observation is also the most relevant procedure in the classification of solid/trabecular patterned tumours, namely in the diagnosis of poorly differentiated carcinoma (PDTC). The Turin proposal for the diagnosis of PDTC has been validated and establishes a useful algorithm in the differential diagnosis of solid/trabecular patterned tumours. Briefly, PDTC are locally invasive and vascular invasive malignant tumours that should not disclose PTC type nuclei. The presence of other features of PDTC, namely convoluted nuclei, high mitotic index and necrosis in the absence of invasion (local and vascular) is clinically irrelevant. Again the use of immunohistochemical markers has not proved to be useful in this setting.
Summing up, we think the use of immunohistochemistry in the study of thyroid tumours is not useful in the diagnosis of PTC, follicular carcinoma or PDTC, nor in the evaluation of vascular or capsular invasion nor in the evaluation of the proliferative index. Besides the research setting, we use the immunohistochemistry in the diagnosis of medullary carcinoma/C cell hyperplasia and in the diagnosis of unusual tumours.
We consider unusual tumours, or tumours that do not 'fit' with the clinical context or those tumours that disclose particular features such as: very high proliferative index (ex. anaplastic carcinoma, angiosarcoma, carcinoma showing thymus like differentiation, metastases in the thyroid), biphasic pattern (ex. metastases occurring within pre-existing primary benign or malignant tumours, mixed medullary-papillary carcinoma, spindle cell tumour with thymus like differentiation) and/or cells without an epithelioid phenotype, namely spindle cell tumours (ex. metastases, spindle cell variant of PTC, hyalinizing trabecular tumour, spindle cell hemangioma, solitary fibrous tumour) and small cell tumours (ex. hematolymphoid lesions, small cell basaloid tumour/carcinoma).

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L 13
Role of the AFAQAP in the accreditation of French anatomo- and cyto-pathology laboratories.

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Introduction

Pathology is subject to a national regulatory framework and, more recently, norms. The regulations, which are obligatory, are under the control of a public administration. Norms are references that aim to harmonise practices to improve quality and safety. While these are not generally obligatory, some norms become obligatory when they are required for a licence, as is the case for ISO 15189 for medical laboratories and very soon for anatomo- and cyto-pathology laboratories in France and Belgium. This reform is happening in many developed countries, but not without some organisational difficulties and resistance from the professionals involved. This resistance, shared by all, is due to the fact that an accreditation process requires a certain amount of time and money and also a long delay for people to adapt. In Belgium, the 5 December 2011 Royal Decree for the licensing of anatomo- and cyto-pathology laboratories provides a clear direction for pathologists. This is not (yet) the case for France.

The French regulatory framework

French law requires medical laboratories to have an ISO 15189 label. This leads to a complicated, evolving situation since medical laboratories are not officially separated from anatomo- and cyto-pathology laboratories.

The *'Hôpital, Patients, Santé, Territoire', (HPST)* law (in English: Hospital, Patients, Health and Territory law) of 21 July 2009 established the reorganization of French healthcare activities. Under article 69 the government was able to reform, using the Ordinance of 13 January 2010, the conditions for the creation, organisation and operation of medical laboratories. This was in response to the 2006 report from the *'Inspection Générale des Affaires Sociales'* (IGAS; in English: Social Affairs General Inspectorate) which noted shortcomings which did not satisfy the public health needs.

This ordinance comprised two main actions: 1) the medicalisation of biological acts, confirming the role of the medical biologist in the health pathway and 2) the accreditation of medical laboratories. Full accreditation of medical laboratories must be completed by 1st November 2016. There are amendments that attempt to push this deadline back to 2018. In France, accreditation is under the responsibility of the 'Comité français d'accréditation' (COFRAC; in English: French Accreditation Committee), which is a unique, independent not-for-profit association.

Because there is no official separation between medical laboratories and anatomo- and cyto-pathology laboratories and some of these laboratories have a legal co-existence, this has enabled the lawmakers to include the anatomo- and cyto-pathology laboratories in the Ordinance, in an ambiguous manner. It is specified in the ordinance that anatomo- and cyto-pathologic examinations carried out using medical biological techniques must be accredited. However, at present, the list of these techniques has not been established and an official text is under discussion to attempt to separate these activities. However, since there is no official position, the situation is unclear.

In this setting, a group of French pathologists set up a working group in 2011, under the authority of the Health Minister, composed of representatives of the different professionals involved, to define their sphere of activities. The document that is being finalised includes the accreditation norm ISO 15189 for anatomo- and cyto-pathology laboratories. The date by which these laboratories should be fully accredited will be at least two years behind that for the medical laboratories. The COFRAC has, at the same time, nominated a group of pathologists to write a specific technical guide for accreditation (TGA) for pathology. The COFRAC is also responsible for training the accreditation inspectors.

In addition the French health care regulations are very strict about formalin, which is considered as a highly dangerous chemical substance (carcinogenic, mutagenic and/or causes infertility), and this biological risk has led to a reorganisation in the private sector since 2010.

The role of the AFAQAP in the accreditation process

The AFAQAP (Association Française d'Assurance Qualité en Anatomie Pathologique; in English: French Association for Quality Assurance in Anatomo-Pathology) is a not-for-profit association created in 1990 by the National Union of French pathologists. Following their independence from the Union in 2000 the association now has a management board with equal representation from the public and private sectors and also representatives of various learned societies and the main professional bodies.

The AFAQAP is involved at various steps in the accreditation process by:

- providing a library of the regulations essential for the efficient operation of anatomo- and cyto-pathology laboratories;
- introducing a manual of good practice guidelines for anatomo- and cyto-pathology: RBPACP v2 (2009) – updated in 2012 with a text on organisation for a diagnosis involving several pathologists;
- drafting the auto-evaluation guidelines for laboratory organisation in collaboration with the HAS (Haute Autorité de Santé; similar to the NICE in the UK);
- organizing post-graduate training on various aspects of the standards, e.g. validation of immunohistochemical (IHC) methods;
- facilitating the internal quality control processes by providing data collection tools (e.g. HER-France, Diag-Inter, Qual-In);

- proposing external quality evaluation for various techniques (IHC, ISH, PCR, etc.), lesion diagnoses (histopathological and cytopathological) and structural organisation.

The regulatory evolution encouraged AFAQAP, at the end of 2011, to undertake a process of accreditation for ISO 17043 which governs providers of proficiency testing schemes.

Conclusion

The challenge for the pathologists and those who work with them will be to come out as winners from a difficult test that is socially important and intellectually interesting, but cost-ineffective and psychologically undermining. This will be possible if they are well prepared and guided. It is a long (about 10 years) but unavoidable road.

L 14

HPV-associated squamous cell carcinoma in the head and neck region

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Squamous cell carcinoma (SCC) represents the most common malignancy of the upper aerodigestive tract. In the oropharyngeal region, SCC is now recognized as being highly associated with the presence of human papillomavirus (HPV) infection. As smoking has declined, the proportion of head and neck SCCs attributable to HPV infection has increased, as has the absolute incidence of these tumors. The presence of HPV infection carries prognostic significance as HPV-associated SCC of the oropharynx has been clearly demonstrated to be associated with improved clinical outcome.

With increased recognition of HPV-associated SCC, the morphologic spectrum of HPV associated malignancy in the oropharynx also has expanded. These tumors typically resemble tonsillar crypt epithelium with basaloid morphology, but also may be well differentiated and keratinizing. In addition, most papillary SCCs of the oropharynx are HPV-related. Uncommonly, HPV associated SCC of the oropharynx exhibits morphologic features that significantly overlap with nasopharyngeal carcinoma of the non-keratinizing type. This variant is primarily significant for the difficulty in distinguishing it from nasopharyngeal carcinoma in the context of lymph node metastasis, since clinical behavior does not differ from HPV-associated SCC with more typical morphology. Rare HPV-associated adenosquamous carcinomas of the oropharynx have also been reported. Most recently, HPV-associated high-grade neuroendocrine/small cell carcinoma of the oropharynx has also been described. Recognition of small cell carcinoma of the oropharynx appears to be clinically important as these tumors appear to have a more aggressive clinical course than other HPV associated head and neck carcinomas.

This talk will provide an overview of the changing epidemiology of head and neck SCC. Histologic features of HPV associated SCC in the head and neck region will be reviewed as will the nomenclature for these tumors. The advantages and disadvantages of various algorithms of testing for HPV (p16 immunohistochemistry, HPV in situ hybridization, and/or HPV PCR) will also be addressed as will the role of such testing as a potential diagnostic tool for the practicing surgical pathologist. Finally, both the prognostic and treatment implications for HPV associated disease will be discussed.

L 15

DRUG-induced side effects in the skin

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According to literature, cutaneous drug eruptions are one of the most common types of adverse reaction to drug therapy, with an overall incidence rate of 2–3% in hospitalised patients.
Although most drug-related skin eruptions are not serious, some of them can be potentially life-threatening. These data stress the importance of the clinical and histological recognition of drug reactions and the need to highlight them.

Drug reactions of the skin are covering a broad range of lesions comprising functional reactions and tumoral lesions, both benign and malignant. Functional reactions are most frequently seen and include many different reaction patterns i.e. acneiform eruptions, hair disorders (alopecia, hypo- and hypertrichosis), drug hypersensivity syndromes, eczematous and exanthematous eruptions, exfoliative dermatitis or erythrodermia, fixed drug reactions, granulomatous drug reactions, lichenoid eruptions, lupus-like adverse drug reactions, lymphoproliferative disorders, nail changes, mucocutaneous lesions, photosensivity, pigmentary disorders, psoriasis, purpura, scleroderma-like reactions, urticaria, vascular reactions, panniculitis and vesiculo-bullous reactions. Importantly, most of these reaction patterns are not caused by a single agent, but often occur in response to various drugs. Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are examples of very severe adverse reactions (SCAR) that are potentially life-threatening.
Tumoral lesions i.e. keratoacanthoma or squamous-cell carcinoma are not infrequently reported in connection with treatment of melanoma with BRAF-inhibitors.

Cutaneous drug reactions can be caused by one drug or may be provoked by several drugs. The latter are referred to ‘’drug reactions caused by drug interactions” that occur when the effects of one drug are modulated by the presence of another drug. Risk factors for the occurrence of drug interactions are polypharmacy in elderly, major organ dysfunction, metabolic factors, social and functional factors, and genetic predisposition.

The precise mechanism of the development of drug reactions is often unknown. The pathogenesis of these skin reactions may be either immunological (i.e. in particular T-cell mediated) or non-immunological. The former are the result of a hypersensitivity reaction with an underlying abnormal immune response. Skin reactions as a result of non-immunological causes are more common and include cumulative toxicity, overdose, photosensitivity, drug interactions, and metabolic alterations. Most drug interactions are pharmacokinetic and involve altered hepatic metabolism by inducing or inhibiting the cytochrome P450 (CYP) isoenzyme system.

Any medicine can induce skin reactions, and certain drug classes, such as non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics and antiepileptics, have drug eruption rates approaching 1–5%. Only few commonly used drugs e.g. cyclosporine, antifungals, erythromycin, sulfonamids and rifampicin often cause side effects. Dangerous combinations are azathioprine (used in the treatment of systemic lupus) in combination with allopurinol and tetracyclines in combination with retinoids.

The BRAF inhibitor vemurafenib produced improved rates of overall and progression-free survival in patients with previously untreated melanoma with the BRAF V600E mutation, but common adverse skin events associated with vemurafenib such as rash, alopecia and photosensitivity have been frequently reported; moreover, keratoacanthoma-like lesions or squamous-cell carcinoma may occur during treatment with BRAF-inhibitors.

In conclusion, side effects in dermatology are a frequent event and may have serious consequences for patient health care. Manufacturers of drugs may have a tendency to minimalise the significance or severity of drug reactions because of their investment of years of, but reporting and publication of drug reactions data by monitoring agencies is needed. Clinicians should keep in mind that cutaneous drug reactions should be suspected in any patient who develops a rash during a course of drug therapy. Dermatologists should keep in mind the few commonly used drugs that often cause drug interactions and be aware that food and health supplements and herbs can participate in such interactions. Monitoring suggestions for dosing regimens and laboratory monitoring should be available to help dermatologists to safely implement systemic drugs in their practice.
Dermatopathologists should be aware of the existence of drug reactions and the fact that these reactions may mimick all histological reaction patterns. Finally and most important, patients should be informed of the possible side effects of drugs and, if possible, encouraged to stop these drugs when skin disorders appear.

L 17
Molecular classification of gliomas

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According to their mode of spreading two main glioma subtypes can be distinguished: circumbscribed gliomas and diffuse gliomas. Pilocytic astrocytoma (PA) belong to the first group. It is the most frequent glioma in childhood, can be cured by total surgical excision and is most often classified as grade I (WHO classification). In contrast, diffuse gliomas mainly occurred in adults and are classified into different subtypes according to their presumptive cell of origin (astrocytomas, oligodendrogliomas and mixed gliomas) and grade (II, III and IV).
Major advances have been made in the front of molecular characterization of glioma subtypes and some markers can be now used in routine practice to increase diagnostic accuracy and to predict outcome (for review Riemenschneider et al 2010).
Recent studies have shown a central role for the mitogen-activated protein kinase (MAPK) pathway in the tumorigenesis of PA. Two major alterations have been recorded in PA. The most frequent is a fusion between *KIAA1549* and *BRAF* arising from a tandem duplication within 7q34. The other is a *BRAF* missense mutation of the V600E type. *KIAA1549-BRAF* fusion mainly occur in posterior fossa PA, whereas V600E *BRAF* mutation, also encountered in pleiomorphic xanthoastrocytomas and gangliogliomas, characterized supra-tentorial PA (Schindler et al 2011).
In adut diffuse gliomas, the major advance was the discovery of *IDH* (isocitrate deshydrogenase) mutations in low grade gliomas (LGG, grade II), anaplastic gliomas (grade III) and secondary glioblastomas (grade IV, Yan et al 2009). *IDH1* mutation occurred at codon 132 and the major mutation is R132H, whereas *IDH2* mutation occurred at codon 172 and is a rare event. The anti-R132H antibody that can be used in formalin-fixed paraffin-embedded specimen constitutes an invaluable help in routine practice (Capper et al 2009). In contrast to LGG, grade III and secondary glioblastomas, primary glioblastomas do not carry *IDH* mutation. In addition, lack of *IDH* mutation (*IDH*- gliomas) has been reported in gliomatosis cerebri and in some LGG of dismal prognostic (Seitz et al 2011, Metellus et al 2010)
Before the discovery of *IDH* mutations, two recurrent molecular alterations were known in LGG: *TP53* mutations and 1p19q codeletion (as the result of t (1;19)(q10;p10) translocation, Jenkins et al 2006). *TP53* mutations (or p53 expression recorded as p53+) characterized astrocytomas whereas 1p19q codeletion (designed as 1p19q+) is a hallmark of oligodendrogliomas.
According to the three major genetic alterations recorded in adult LGG, four molecular groups can be set up: group 1 IDH+/p53-/1p19q-, group 2 IDH+/p53-/1p19q+, group 3

IDH+/p53+/1p19q- and group 4 triple negative gliomas (Figarella-Branger et al 2011). These groups are designed because 1) 1p19q codeletion and p53 mutation are mutually exclusive (rewieved in Riemenschneider et al 2010 2) *IDH1* mutation preceeds *p53* mutation or 1p19q codeletion (Watanabe et al and 3) triple negative gliomas exist (Metellus et al 2010).
Interestingly, by using this classification we were able to classify all LGGs. From the study performed by Kim et al 2010 we also learnt that 93% of LGGs were accurately classified into these four groups, and the percentage of cases recorded in each subgroups remained in the same range. This molecular classification predicts overall survival on uni- and multivariate analysis (P=0.001 and P=0.007 respectively).
Taken together we strongly recommend to search for R132H expression in all diffuse gliomas for diagnostic and prognostic relevance because it has been reported that R132H positive gliomas have a better prognostic than their negative counterparts and thus whatever the grade (Sanson et al 2009). When negative, search for minor *IDH1* and *IDH2* mutation is recommended in grade II and III gliomas to be able to classify them as IDH+ or IDH-. In these gliomas search for p53 expression and especially 1p19q deletion is of importance to better classify them. Moreover 1p19q codeletion is predictive of treatment response. In all cases it is of importance to correlate pathological features with imaging to rule out gliomatosis cerebri or an infiltrative part of a glioblastoma. Because glioblastomas often display EGFR amplification (often associated with strong and diffuse expression, Coulibaly et al 2010) search for this genetic alteration might be of diagnostic interest but its prognostic relevance is a matter of controversy.

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L 19

Training and education of Cytotechnologists in Europe

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The screening programs against cervical cancer are changing in many countries in Europe. HPV-testing, liquid based cytology and automation have been implemented in many laboratories in Europe and HPV vaccination in several national vaccination programs. These changes have an impact on the Cytotechnologists profession. In some countries a screening algorithm with HPV as primary screening and cytology as triage to HPV positive women is being discussed. We know that the future of our profession will change and it is important that the training programs adapt to these changes. What basic education do we need before entry into cytology training? How do we maintain competence in cervical cytology when the number of cytology specimens decreases dramatically, when HPV-testing is used as primary screening method, and the prevalence of low and high-grade lesions decreases as the vaccinated women reaches screening age? We know that training programs in cytology varies among the European countries as well as the acknowledgement of Cytotechnologists. How can we help Cytotechnologists to be recognized at a European level? It is useful to chart the present situation of training and education of Cytotechnologists in Europe in order to know what to plan for the future.

In 2010 EFCS, represented by the Secretary General Philippe Viehl, gave three assignments to members of the European Advisory Committee of Cytotechnology (EACC):
1. To update the overview of Training and Education of Cytotechnologists in Europe from 2006 (http://www.eurocytology.eu/www.eurocytology.eu - training package 3- module 4).
2. A summary of what is common and what is different in the European countries.
3. Propose guidelines for minimum requirements for practising cytotechnology in Europe.

A questionnaire was distributed in 2010 to 21 countries (14 countries with an EACC member and 7 countries with an EFCS member, but no EACC representative). We did not succeed in reaching nine other countries with an EFCS member according to the membership list at www.efcs.eu. We received answers from 14 countries (13 with an EACC representative and one without).
The questionnaire was similar to the one from 2006, besides adding a couple of questions concerning optimal education. The preliminary results were presented at the EFCS meeting at the International Congress of Cytology in Edinburg in 2010. It was decided at the meeting that we should extend and refrase some of the questions and hopefully receive more answers. The new, updated and extended questionnaire was distributed in spring 2011 to 25 countries (14 countries with an EACC member and 11 countries with an EFCS member, but no EACC representative). We received

answers from 18 countries (14 countries with an EACC member and 4 without). Three countries were excluded from the survey because they either lack training programs in cytology or they do not have Cytotechnologists. The questionnaire dealt with questions concerning number of fully trained and employed Cytotechnologists, competence level, basic education before entry into cytology training, training in cytology, accreditation, certification, continuing education, quality assurance, and questions about optimal education. The results were presented at The European Congress of Cytology in Istanbul, Turkey in 2011 and will be presented on 21.April at the Belgian Week of Pathology 2012. A brief summary is given here:
Basic education before entry into cytology training is mostly medical laboratory technologist or biomedical scientist with a bachelor degree. The majority of Cytotechnologists are on intermediate competence level, which is enabling them to sign out normal and inadequate cervical specimens, undertake rescreening and offer a differential diagnosis on abnormal specimens. The duration of training in cytology varies from 3 months to 2 years. The number of cervical specimens screened with supervision in training varies from 700 – 7000, but mostly around 1000 cervical specimens. The training is given as in-house training in the cytology laboratory, separate course or both. Accreditation of the training in cytology is usually from an academic institution and the requirements are mostly decided by the professional societies. Most of the respondents believe that a certified diploma from the EFCS would enhance the acknowledgement of Cytotechnologists in Europe. A presentation of the results is also available on the EACC website: www.efcs.eu - EACC

The members of EACC will now discuss and propose minimal requirements for practising cytotechnology in Europe. The proposal of guidelines will be presented at the next EFCS council meeting at the European Congress of Cytology in Cavtat/ Dubrovnik in Croatia 30. Sept. 2012. The participants at the Belgian Week of Pathology are encouraged to contribute to the discussion.

I will also present the basic requirements for practicing cytotechnology in Norway as an example of how it could be done. Basic education before entry into cytology training is bachelor in biomedical science. The bachelor program encompass among other subjects: molecular biology, cell biology, biochemistry, introduction to gynae and non-gynae cytology. When employed in the cytology laboratory, the trainees follow a structured in-house training program. The trainees must screen 1000 cervical specimen with supervision and pass an internal test before they are allowed to sign out normal and inadequate cervical specimens. The training in non-gynae cytology, begins after this period. After at least two years of employment it is mandatory to attend a postgraduate education program in clinical cytology. The postgraduate program in clinical cytology (30 cp) is administrated by Sør-Trøndelag University College. The lecturers, both experienced Cytopathologists and Cytotechnologists, are recruited from the members of the Norwegian Society of Clinical Cytology. The program is a combination of self-study following a curriculum, internal education at the cytology laboratory and two one week sessions at Sør-Trøndelag University

College. The first session covers: cervical cytology, cell biology, molecular biological methods, staining methods and QA/QC. The second session covers non-gynae cytology (respiratory tract, urinary tract and body cavities), special staining, and immunocytochemistry. The employees are granted paid leave when they attend the two week sessions at the University College. Every 3 to 4 years it is mandatory to attend a refresher course in cervical cytology with emphasis on cytological criteria (18 hours) at Sør-Trøndelag University College.

Which roles will the Cytotechnologist have in future Cytopathological diagnostics and what skills are necessary to meet the future needs? The clinicians ask for more than a diagnosis. They want help with designing therapy and predicting prognosis. Molecular cytology is an emerging discipline. Cytological specimens are well suited for analyses at the molecular level. This is a field for Cytotechnologists with special interest in molecular biology and molecular diagnostic techniques.

Cytotechnologists are passionate about their work, responsible, intelligent problem solvers, highly trained morphologists and technologists. We know from previous surveys in Europe that Cytotechnologists in general, perform much more than screening cervical cytology specimens. They evaluate non-gynae specimens, perform HPV-testing, immunocytochemistry, FISH, teaching, biobanking, QA/QC, assists in on site assessment of FNAC, EBUS or imprint etc.

My belief is that Cytotechnologists will play an important role in the future Cytopathological diagnostics. It is my personal opinion that the prerequisite is basic education as bachelor in Biomedical Science or similar before entry into cytology training and ancillary techniques. The future Cytotechnologist has to be multiskilled and flexible to meet future needs. The training and education programs in cytotechnology must adapt to the changing roles of Cytotechnologists. To succeed in this work, each country has to have an organized training and education program in cytotechnology, recognized on a national level.

L 20

HPV-associated squamous cell carcinoma in the head and neck region

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Squamous cell carcinoma (SCC) represents the most common malignancy of the upper aerodigestive tract. In the oropharyngeal region, SCC is now recognized as being highly associated with the presence of human papillomavirus (HPV) infection. As smoking has declined, the proportion of head and neck SCCs attributable to HPV infection has increased, as has the absolute incidence of these tumors. The presence of HPV infection carries prognostic significance as HPV-associated SCC of the oropharynx has been clearly demonstrated to be associated with improved clinical outcome.

With increased recognition of HPV-associated SCC, the morphologic spectrum of HPV associated malignancy in the oropharynx also has expanded. These tumors typically resemble tonsillar crypt epithelium with basaloid morphology, but also may be well differentiated and keratinizing. In addition, most papillary SCCs of the oropharynx are HPV-related. Uncommonly, HPV associated SCC of the oropharynx exhibits morphologic features that significantly overlap with nasopharyngeal carcinoma of the non-keratinizing type. This variant is primarily significant for the difficulty in distinguishing it from nasopharyngeal carcinoma in the context of lymph node metastasis, since clinical behavior does not differ from HPV-associated SCC with more typical morphology. Rare HPV-associated adenosquamous carcinomas of the oropharynx have also been reported. Most recently, HPV-associated high-grade neuroendocrine/small cell carcinoma of the oropharynx has also been described. Recognition of small cell carcinoma of the oropharynx appears to be clinically important as these tumors appear to have a more aggressive clinical course than other HPV associated head and neck carcinomas.

This talk will provide an overview of the changing epidemiology of head and neck SCC. Histologic features of HPV associated SCC in the head and neck region will be reviewed as will the nomenclature for these tumors. The advantages and disadvantages of various algorithms of testing for HPV (p16 immunohistochemistry, HPV in situ hybridization, and/or HPV PCR) will also be addressed as will the role of such testing as a potential diagnostic tool for the practicing surgical pathologist. Finally, both the prognostic and treatment implications for HPV associated disease will be discussed.

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L 22

Spindle cell tumors: a practical morphological, immunohistochemical and molecular approach

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Spindle cell tumors are a very large and heterogenous group of tumors that can occur in many different locations and have morphologic similarities. The differential diagnosis of monomorphic spindle cell sarcomas often includes monophasic spindle cell synovial sarcoma (1,2), malignant peripheral nerve sheath tumor (MPNST) (3,4), malignant solitary fibrous tumor (5,6) and leiomyosarcoma. Less common in this group of spindle cell sarcomas are low-grade fibromyxoid sarcoma (7,8,9,10), low-grade myofibroblastic sarcoma (11), embryonal and spindle cell rhabdomyosarcoma (mostly occurring in children and young adults) (12,13,14) and infantile fibrosarcoma (infancy) (15). Extremely rare examples of spindle cell sarcomas are the adult-type fibrosarcoma, sclerosing epithelioid fibrosarcoma (10), follicular dentritic cell sarcoma (16) and intimal sarcoma (17,18). The diagnosis of fibrosarcoma is a diagnosis of exclusion and should be made only by failure to identify specific differentiation and/or genetic abnormality. With the multiple immunohistochemical and molecular techniques now available, spindle cell sarcomas with fascicular pattern are nearly always diagnosable as synovial sarcoma, MPNST, leiomyosarcoma or other specific sarcoma subtype. Thus, unspecified types of fibrosarcoma in adults have become increasingly rare and difficult to define. Spindle cell mesenchymal tumors that are encountered most frequently within the abdomen or retroperitoneum are dedifferentiated liposarcoma (19), leiomyosarcoma, gastrointestinal stromal tumor (GIST) (20), desmoid fibromatosis (21) and inflammatory myofibroblastic tumor (22). Some spindle cell sarcomas have a predominantly myxoid appearance (e.g. low grade myxofibrosarcoma, myxoinflammatory fibroblastic sarcoma, extraskeletal myxoid chondrosarcoma) (see session myxoid tumors). The group of spindle cell tumors also includes a lot of mimickers of spindle cell sarcoma, like non sarcomatous malignant tumors (spindle cell "sarcomatoid" carcinoma (23), spindle cell melanoma (24)) and a very large group of benign soft tissue lesions which mimic sarcoma like pseudosarcomatous (myo) fibroblastic tumors (nodular fasciitis (25), intravascular and cranial fasciitis, postoperative spindle cell nodule, visceral reactive myofibroblastic lesions (26),…), nerve sheath tumors (bizarre neurofibroma, cellular schwannoma (27), ancient schwannoma,…) and fibrohistiocytic lesions (cellular fibrous histiocytoma (28), atypical fibrous histiocytoma (29), atypical fibroxanthoma (30), …). It should be remembered that in adults carcinoma and melanoma are more common than sarcomas and should always be ruled out before making the diagnosis of a spindle cell sarcoma especially if there is a previous history and in mucosal or organ-based locations including the skin (31), aerodigestive tract, kidney (32,33,35), bladder (34), female genital tract (36), breast (37,38), lung (39),…. Correlation with clinical data (clinical history, age, tumor site, tumor size) are extremely

important and additional clinical information can be very helpful and crucial to make a correct diagnosis (e.g. previous radiation in postradiation sarcoma; neurofibromatosis type 1 (40) in malignant peripheral nerve sheath tumor). The morphological key features to appreciate a spindle cell tumor are the circumscription (encapsulation, infiltration), the cellularity, stromal features (myxoid stroma, fibrotic/hyalinized stroma, calcifications, formation of metaplastic tissue, multinucleated giant cells), the growth pattern (fascicular, herringbone, hemangiopericytic, palissading, storiform, fibrotic/hyalinizing) and the cytological features (nuclear and cytoplasmic characteristics).

Immunohistochemistry (41,42) is particularly useful adjunct to light microscopy in the diagnosis of spindle cell tumors and in my experience an important if not obligate part of the work-up of certain spindle soft tissue tumors especially in the abdomen/retroperitoneum and skin (43,44). To use immunostains in the most effective and costefficient way, it is useful to have an algorithmic approach in mind and to use these reagents in panels. A proposed screening panel for monomorphic spindle cell tumors consists of Pancytokeratin (MNF 116 or AE1/AE3), S100, CD34, SMA and Desmin. In the context of an appropriate morphology a diagnosis of leiomyosarcoma can be made by the use of desmin, smooth muscle actin and/or Caldesmon. Desmin is highly specific and stains only a number of non-muscular tumors and a few myofibroblastic lesions such as angiomyofibroblastoma. Smooth muscle actin, although less specific and more widely distributed especially in myofibroblastic lesions, is more sensitive for leiomyomatous differentiation and proves positive in 90% of leiomyosarcomas. Myogenin (MYF4) or MyoD1 are useful in the setting of a rhabdomyosarcoma. Although most benign nerve sheath tumors are positive for S-100 protein, this is not true for MPNST which are only positive in 50-60% of cases at most and very focally. Immunohistochemistry is very valuable in the diagnosis of monophasic spindle cell synovial sarcomas, as up to 90% of these cases are epithelial membrane antigen (EMA) positive and from 60-70% express low-molecular weight keratins. It is important to note that a significant proportion of the synovial sarcomas (up to 30%) also show at least focal S100 positivity. Strong CD34 expression is seen in solitary fibrous tumor and dermatofibrosarcoma protuberans (DFSP) and is helpful in distinguishing such cases from monophasic synovial sarcoma and fibrous histiocytoma (FH) respectively (although a minority of cellular FH are positive). In case of a spindle cell tumor in the abdomen MDM2 (45), Beta-Catenine (46) and a CD117 (C-KIT) (47,48) staining can be necessary to support the diagnose of dedifferentiated liposarcoma, a desmoid fibromatosis or a GIST respectively. New developed immunohistochemical markers can be used in difficult cases like DOG1 (GIST) (47,48) and MUC4 (low-grade fibromyxoid sarcoma) (49).

Molecular genetic and cytogenetic testing have become increasingly important diagnostic tools. There are a number of different diagnostic situations where identification of a specific translocation can be an important adjunctive diagnostic tool to immunohistochemical stains, like in the diagnosis of a synovial sarcoma (specific t(X;18) translocation, SYT-SSX fusion) (50,51), especially in poorly differentiated examples of synovial sarcoma, or DFSP (t(17;22) translocation, COL1A1-PDGFB fusion)(52). A correct diagnosis

of these last two types of spindle cell sarcomas may have important therapeutic implications because the chemosensitivity of synovial sarcoma (particularly to ifosfamide) and the sensitivity for a tyrosine kinase inhibitor (such as Gleevec) in case of a (fibrosarcomatous variant of a) DFSP (53). Dedifferentiated liposarcomas usually show the presence of ring or giant marker chromosomes with at the molecular level amplification of MDM2 gene (12q12-15 region) (45). A consistent chromosomal translocation t(7;16) (q34;p11) (FUS-CREB fusion) is seen in low-grade fibromyxoid sarcoma and this is of diagnostic value in paraffin-embedded material (8,9). Infantile fibrosarcoma has a specific translocation t(12;15)(p13;q25) leading to fusion of ETV6 and NTRK3 genes (15).
Electron microscopy, although sometimes still usefull (in schwannian and perineurial tumors-presence of nerve sheath differentiation), is relatively time consuming and requires special fixation.

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L 23
Myxoid tumors of soft tissue

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1. INTRODUCTION.

- Heterogenous group of lesions characterized by an abundance of mucoid extracellular matrix (hyaluronic acid, chondroitin sulfate, keratan sulfate...).
- Myxoid change can be seen, at least focally, in a wide variety of soft tissue neoplasms
- Broad histological overlap, often resulting in the inability to separate benign and malignant lesions.

Table 1. Myxoid tumors of soft tissue

Benign	Malignant
Ganglion cyst Superficial angiomyxoma Dermal nerve sheath myxoma Intramuscular myxoma Juxta-articular myxoma Superficial acral fibromyxoma Deep angiomyxoma Ossifying fibromyxoid tumor Myoepithelioma / Mixed tumor	Myxofibrosarcoma Myxoid liposarcoma Extraskeletal myxoid chondrosarcoma Low-grade fibromyxoid sarcoma Myxoinflammatory fibroblastic sarcoma Myxopapillary ependymoma Chordoma
Myxolipoma Myxoid nodular fasciitis Myxoid spindle cell lipoma Myxoid neurofibroma ...	Myxoid DFSP Myxoid MPNST Myxoid leiomyosarcoma Epithelioid hemangioendothelioma ...

(many other tumors not listed here may display focal myxoid change)

1.1. SAMPLING

- Adequate sampling is mandatory as it will allow the separation between «true» myxoid tumors (intramuscular myxoma, myxoid liposarcoma...) and tumors that occasionnaly display focal myxoid change (myxoid DFSP, myxoid MPNST...). For the latter, the identification of areas with a classical morphology is very important as myxoid change tend to obscure the usual diagnostic features. For example, myxoid DSFP usually loses its typical storiform architecture.

1.2. CLINICAL INFORMATION

*AGE

- Children: lipoblastoma, botryoid RMS, ...
- Young adults: DFSP, myxoid liposarcoma, myxoid nodular fasciitis...
- Elderly patients: myxofibrosarcoma,...

*TUMOR DEPTH

Table 2. Tumor depth

Superficial (dermal, subcutaneous)	Deep (subfascial)
Superficial angiomyxoma	Intramuscular myxoma
Dermal nerve sheath myxoma	Juxta-articular myxoma
Superficial acral fibromyxoma	Deep angiomyxoma
Ossifying fibromyxoid tumor	Myxoid liposarcoma
Myxoid nodular fasciitis	Extraskeletal myxoid chondrosarcoma
Myxoid spindle cell lipoma	Low-grade fibromyxoid sarcoma
Myxoid neurofibroma	Myxoid MPNST
Myxoinflammatory fibroblastic sarcoma	
Myxoid DFSP	
...	...
Myxofibrosarcoma	

*TUMOR SITE

- Distal extremities: superficial acral fibromyxoma (nail bed area), myxoinflammatory fibroblastic sarcoma
- Retroperitoneum: well-differentiated/dedifferentiated liposarcoma
- Perineum: agressive angiomyxoma, angiomyoifbroblastoma...
- Sacro-coccygeal area: myopapillary ependymoma, chordoma

1.3. HISTOLOGICAL FEATURES

Most important clues:

- Circumscribed ? Infitrative ?
- Lobulation ?
- Vascularization ?
- Nuclear atypia

*CIRCUMSCRIPTION / PATTERN OF INFILTRATION

- Myxofibrosarcoma: infiltration +++
- Myxoid liposarcoma: very sharp circumsciption
- OFMT: pseudocapsule with bone trabeculae

*LOBULATION

Table 3. Myxoid soft tissue tumors with a prominent lobular architecture
- Dermal nerve sheath myxoma (lobulation +++, well circumscribed lobules) - Superficial angiomyxoma (lobulation +++, poorly delineated lobules) - Lipoblastoma - Myxofibrosarcoma - ES myxoid chondrosarcoma (lobules separated by fibrous septa) - Chordoma

*VASCULARIZATION

Table 4. Myxoid soft tissue tumors with a prominent and/or typical vascularization:
- Myxoid liposarcoma: thin-walled branching capillaries (“chicken-wire” pattern) - Myxofibrosarcoma: thick-walled curvilinear capillaries - Low-grade fibromyxoid sarcoma: complex vascularization (arches, bridges...), sclerotic vessels

- Poorly vascularized tumors: intramuscular myxoma, ES myxoid chondrosarcoma,...

*LACE-LIKE ARCHITECTURE

- A lace-like or trabecular architecture (anastomosed cords of neoplastic cells) is characteristic of a few myxoid neoplasms: chordoma, extraskeletal myxoid chondrosarcoma, myoepithelioma / mixed tumor, ossifying fibromyxoid tumor

1.4. IMMUNOHISTOCHEMISTRY

- Non-specific for most myxoid tumors
- Useful in 3 settings:
    - Benign nerve sheath tumors (neurofibroma, dermal nerve sheath myxoma): S100+++
    - Myoepithelioma/mixed tumors (S100+, CK+, SMA +/-, GFAP +/-...) and differential diagnosis with chordoma, myxoid chondrosarcoma
    - Low-grade fibromyxoid sarcoma: consistent expression of MUC4

1.5. CYTOGENETICS

Table 5. Genetic alterations associated with myxoid tumors			
Lipoblastoma	*PLAG1* rearr., amplif chr.8		
Myoepithelioma / mixed tumor	Rearr. EWSR1		
Myxoid liposarcoma	t(12;16)(q13;p11) t(12;22)(q-13;p11)	*FUS/DDIT3* *FUS/EWSR1*	(>90%) (rare)

ES myxoid chondrosarcoma	t(9;22)(q22-31;q11-12) t(9;17)(q22;q11-12)	*EWSR1/NR4A3* *RBP56–NR4A3*	(70%) (rare)
Low-grade fibromyxoid sarcoma	t(7;16)(q32-34;p11) t(11;16)(p11;q11)	*FUS-CREB3L2* *FUS-CREB3L1*	(>75%) (rare)
Myxoinflammatory fibroblastic sarcoma	t(1;10)(p22;q24)		
Myxoid DFSP	t(17;22)(q22;q13)	COL1A1-PDGFB	
Well diff./dédiff. liposarcoma	rearr. 12q14-15	amplif. *mdm2, cdk4...*	

2 MYXOID TUMORS OF SOFT TISSUE (selected entities)

2.1. Ganglion cyst
- Common
- Myxoid changes can be prominent and simulate a myxoid neoplasm (juxta-articular myxoma, low-grade myxofibrosarcoma...)

2.2. Superficial angiomyxoma
- Rare
- Syn.: "cutaneous myxoma"
- Superficial
- Wide age range
- Small papules / polypoid lesions
- Head and neck area, trunk, genital area
- **Lobulation +++ (poorly demarcated lobules)**
- Spindled or stellate fibroblasts, small capillaries, **neutrophils**
- Entrapped adnexal structures in 1/3 of cases
- IHC: non specific (CD34+/-)
- DD: Dermal nerve sheath myxoma, low-grade myxofibrosarcoma
- Benign, recurrences 30-40%
- **Multiple lesions or external ear lesion: rule out Carney complex ! (cutaneous pigmented lesions, endocrine abnormalities, cutaneous myxomas, cardiac myxomas)**

2.3. Dermal nerve sheath myxoma
- Rare
- Syn.: "myxoid neurothekeoma"
- No relationship with cellular neurothekeoma
- Superficial
- Young adults adults
- Extremities > trunk , H/N area
- **Lobulation +++ (sharply circumscribed lobules)**
- Elongated cells, nuclei grouped in clusters
- IHC: **S100+++**
- DD: Superficial angiomyxoma, neurofibroma
- Benign, rare recurrences

2.4. Superficial acral fibromyxoma
- Relatively common
- **Distal extremities, nail bed area +++**

- Spindled or stellate fibroblasts, storiform / short fascicles, abundant mast cells
- IHC: CD34 +, EMA +/-, CD99 +/-
- Benign

2.5. Intramuscular myxoma
- Adults
- Limbs, proximal
- Deeply seated
- Hypocellular, spindled / stellate fibroblasts, poor vascularization, no atypia
- IHC: non spécific
- Mazabraud Syndrome: IM myxoma and fibrous dysplasia
- Benign

2.6. Juxta-articular myxoma
- Adults
- Deeply seated
- Near larger joints
- Often more cellular than IM myxoma, no atypia
- Benign, recurrences: 1/3 of cases

2.6. Deep angiomyxoma
- Rare
- Perineum
- Mostly females, rare cases in males
- 30-50 yo
- Poorly circumscribed
- Low cellularity, stellate / spindle fibroblasts, hyalinized vessels
- Recurrences: 10-30% of cases, no metastases

2.7. Ossifying fibromyxoid tumor
- Rare
- Young adults
- Subcutaneous, Limb girdles / trunk
- Well circumscribed, fibrous pseudocapsule, **rim of lamellar bone (90%)**
- Myxohyaline stroma, bland ovoid/polygonal cells, **lace-like architecture**
- IHC: S100 +/- (70%), desmin +/- (40%)
- Usually benign
- Increasing cellularity, mitoses, atypia: malignant OFMT (locally agressive, metastases)

2.8. Myoepithelioma / mixed tumor
- Wide age range
- Extremities, superficial or deep-seated
- **Resemble myoepithelioma / mixed tumor of salivary glands**
- **IHC: CK + (90-95%), S100 + (90%), EMA +/- (40%), GFAP +/- (30%), SMA +/- (20%), desmin +/- (15%), p63 +/- (15%)**
- CG/MB: **translocations involving *EWSR1* and other genes**
- Usually benign: recurrences 15-20%
- High grade arypia = malignant; agressive behavior with metastases

2.9. Lipoblastoma
- **Children, < 10 yo**
- Subcutaneous or deep seated
- Limbs (proximal), trunk, retroperitoneum, mediastinum
- **Lobulation**
- "Mature", resembling lipoma, or "immature", resembling myxoid liposarcoma
- CG/MB: ***PLAG*1 rearr.** , amplif. chr.8

2.10. Other benign myxoid tumors
- spindle cell lipoma
    - males >40 yo
    - Neck, upper trunk, usually superficial
    - Rare cases with extensive myxoid change, responsible for a "pseudolymphangiomatous" pattern
- nodular fasciitis, neurofibroma, ...

2.11. Myxofibrosarcoma
- **Relatively common**
- Elderly patients
- Limbs +++ (thigh)
- Usually superficial (70%)
- Poor demarcation, **very infiltrative**
- **Lobulated (lobules with varying cellularity)**
- Fibroblast-lie cells with **nuclear hyperchromasia**, pseudolipoblasts
- **Abundant curvilinear vessels**
- rare cases with epithelioid morphology
- IHC: non specific
- CG/MB: complex caryotype
- Frequent recurrences, distant metastases for intermediate/high-grade lesions

2.12. Myxoid liposarcoma
- Young adults
- Limbs +++, never intraabdominal
- Deep seated
- Well circumscribed, **sharp margins**
- **"Crow's feet" vasculature, mucin pools**, variable cellularity**,** small univacuolated lipoblasts (periphery)
- IHC: non specific
- CG/BM**: t(12;16)(q13;p11)** ***FUS/DDIT3*** **or t(12;22)(q-13;p11)** ***FUS/EWSR1***
- High grade («round cell areas»): poor prognosis, with pleural / peritoneal metastases

2.13. Extraskeletal myxoid chondrosarcoma
- Adults
- Limbs
- Deep seated
- **Lobulation +++ with fibrous septa**
- **Lace-like architecture**
- **Poorly vascularized**
- IHC: non specific (but may be useful in the DD with mixed tumor/myoepithelioma)

- CG/MB: t**(9;22)(q22-31;q11-12)** ***EWSR1/NR4A3*** or variants: t(9;17)(q22;q11-12)
- Local recurrences, late metastases

2.14. Low-grade fibromyxoid sarcoma

- Young adults
- Usually deep seated-
- **Alternance of myxoid and fibrous areas**
- Spindle-shaped cells with **minimal atypia**
- **Complex capillary network; perivascular sclerosis**
- rare cases with collagenous pseudosettes, rare epithelioid foci
- **IHC: MUC4+**, EMA +/-
- CG/MB**: t(7;16)(q32-34 ;p11)** ***FUS-CREB3L2*** or rarely t(11;16)(p11;q11)
- Delayed metastases in up to 50% of cases

2.15. Myxoinflammatory fibroblastic sarcoma

- Adults
- **Hand/wrist, feet/ankle**
- Alternance of myxoid areas and collagenous/inflammatory areas
- Mixed inflammatory infiltrate, **atypical cells with prominent "virocyte-like" eosinophilic inclusions**
- IHC: non specific
- CG/MB: t(1;10)(p22;q24) with rearrrangements of *TGFBR3* and *MGEA5*
- Frequent recurrences (up to 2/3 of cases), very rare metastases

2.16. Other malignant myxoid tumors

- Well-differentiated/dedifferentiated LS:
  - Most common retroperitoneal sarcoma; **should always be included in the DD of retroperitoneal/intraabdominal myxoid sarcomas**
  - **IHC: mdm2 +, cdk4 +**
  - CG/MB: amplification of 12q13-21
- Myxoid DFSP:
  - Myxoid changes are usually focal.
  - The typical storiform architecture of DFPS is usually lost
- Myxoid MPNST, myxoid leiomyosarcoma, epithelioid hemangioendothelioma, ...

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L 25

Small blue round cell tumors.

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The term small blue round cell tumors is frequently used but is in fact partially a misnomer. The tumor cells are usually not small, but they represent malignant tumors composed of undifferentiated cells with a rather big round nucleus and a minimal amount of cytoplasm. Because of their morphological overlap, careful correlation with the clinical data and additional techniques are almost always necessary to define their true nature. Immunohistochemistry usually is sufficient, but luckily, the majority of these lesions have well defined cytogenetic changes which represent a diagnostic tool. The fusion transcripts can be detected by RT-PCR, and the genetic breakpoints by FISH on paraffin sections.
In this review, only the most frequent mesenchymal small blue round cell tumors will be discussed. It is clear that also non mesenchymal tumors can present with this phenotype, like lymphoma, leukemic deposits, (small cell) carcinoma, melanoma.

Rhabdomyosarcoma

Rhabdomayosarcomas are primitive malignant tumors that show an incomplete differentiation towards skeletal muscle. Two subgroups can present as small blue round lesions, the embryonal (ERMS) and alveolar rhabdomyosarcoma (ARMS). It is important to differentiate between these entities because ERMS has a much better prognosis than ARMS. The 5 year survival of ARMS is 45%, in ERMS it goes up to 70% (1).
ERMS is the most frequent sarcoma of childhood, and it represents 70 to 75% of all rhabdomyosarcomas. Children between 5 and 15 years are typically affected (45%), while up to 35% occur before the age of 5. The head & neck region is the predominant site of involvement (+/- 47%), orbit, eyelid, oropharynx, parotid gland, auditory canal, middle ear, pterygoid fossa, nose, paranasal sinuses, tongue and cheek being typical sites. The genitourinary system is involded in +/- 28% of cases, the urinary bladder, prostate and paratesticular soft tissues as typical examples. Involvement of skeletal muscle, visceral organs, retroperitoneum, pelvis and perineum is rare. In its typical presentation, ERMS contains a mixture of primitive spindle to spiderlike mesenchymal cells and rounded cells with eccentric cytoplasm, corresponding to rhabdomyoblasts. The intercellular matrix may be sparse or abundant and myxoid.
ARMS usually affects a slightly older population, children/young adults between 2 and 25 years are mainly affected. These tumors account for only 20 to 25% of all rhabdomyosarcomas and mainly occur in the skeletal muscle of the extremities, additional sites of involvement are head & neck, trunk and pelvis. The *conventional* type of alveolar rhabdomyosarcoma is characterized by fibrovascular septa that

separate the tumor into nests. In the center of these discrete nests, the cells are dischohesive, while the cells at the edge are attached to each other and to the septa, simulating the alveolar pattern of the lung. The tumor cells are relatively large, the largest ones (15 to 30 micrometer) often showing rhabdomyoblastic differentiation with eccentric eosinophilic cytoplasm, with/without cross striations. The majority of the cells is less differentiated with a round nucleus and little cytoplasm. Multinucleated giant ('wreath cells') are often present as well and are of help in the (differential) diagnosis. In the diagnostically more challenging *solid* variant of alveolar rhabdomyosarcoma, the fibrovascular septa are hardly found and there is no discohesive growth pattern. The tumor cells show the same morphology as in the conventional variant.

In addition to the clinical features, a number of microscopic characteristics help to differentiate between ERMS and ARMS. In ARMS the tumor cells are usually larger than in ERMS. Both should be positive for desmin and myogenin (nuclear!), but the amount of myogenin positive cells is usually musch higher in ARMS than in ERMS. Finally, ARMS exhibits a characteristic t(2;13)(q35;q14), or a t(1;13)(p36;q14) as variant translocation. In ERMS a consistent diagnostic cytogenetic abnormality has not been described. Structural and numerical changes are quite frequent, including extra copies of chromosomes 2, 8, and 13. Allelic loss in chromosomal region 11p15 has also been described (2).

EWING sarcoma (ES)/Primitive Neuroectodermal Tumor(PNET)/Askin Tumor

Formerly, these three names covered different entities, but insight into the molecular changes has shown that they belong to a phenotypic spectrum of one and the same entity, usually labeled as ES/PNET. Most patients are adolescents or young adults younger than 30 years, and ES/PNET is the second most common pediatric sarcoma. Skeletal ES/PNET can occur anywhere, but especially in long bones. Extraskeletally, it may arise at virtually every site, but common regions include extremity deep soft tissue, the paravertebral area, retroperitoneum and thoracopulmonary region (Askin tumor). Parenchymal and skin presentations have also been described. Patienst with localized disease can achieve up to 90% 5 year survival, but this falls up to 30% when metastases are present at diagnosis. On histology ES/PNET usually differs from rhabdomyosarcoma in that it is a uniformly round cell proliferation, often with a clear cytoplasmic rim, due to the presence of glycogen, which can be highlighted by a PAS/PAS-α stain. Diffuse membrane positivity for CD99, usually in a honeycomb pattern, is present in the vast majority of ES/PNET. It should be noted that CD99 is a very sensitive but not very specific marker for ES/PNET. About 70% of cases show nuclear positivity for FLI1. The fact that a minority of ES/PNET can express keratin and occasionally desmin can be confusing for the differential diagnosis with synovial sarcoma and rhabdomyosarcoma. All ES/PNET are characterized by recurrent chromosomal translocations resulting in EWS-ETS family fusion oncoproteins. The most common is t(11:22)(q24;q12) with a EWSR-FLI1 fusion, some 10% of tumors harbour a t(21;22)(q22;q12) with EWS-ERG fusion (3-5).

Desmoplastic small round cell tumor (DSRCT)

This is a rare but very aggressive small blue round cell tumor that poorly responds to any therapy. DSRCT usually affects children, adolescents and young adults, with a male predominance. The abdomen and pelvis are usually involved, often with multifocal spread at presentation (6). Occurrence at other sites is rare, but well described. We recently saw a genuine case in the wall of the oesophagus. As ES/PNET, DSRCT usually is a rather uniform round cell tumor, but the cells are most often packed in nests, in a dense desmoplastic stroma. Importantly, some cases are not 'small blue round', and some tumors do not have the typical desmoplastic stroma, which challenges the diagnosis. This tumor is of uncertain lineage, but shows polyphenotypic differentiation, including co-expression of keratin, desmin (both often in a dot-like fashion), and neural markers like synaptophysin, chromogranin or neurofilament. Myogenin is absent, as is CD99 in the majority of cases. DSRCT is typically positive for WT1. This corresponds to the diagnostic t(11;22)(p13;q12) with formation of the EWSR1-WT1 fusion gene (7-9).

Neuroblastoma

Neuroblastoma is the third most common malignancy in childhood after hematolymphoid and central nervous system neoplasms. The peak age of demonstration is +/- 18-21 months, and most cases are diagnosed by 5 years.
The adrenal medulla, or any location along the sympathetic ganglia chain is most frequently involved. The prognosis depends on many factors, including age, staging and histology. The low power view of neuroblastoma usually shows a multilobular tumor with frequent calcifications. The cells have uniform rounded nuclei, the matrix shows a typical fibrillar pattern (neuropil). Rosette formation can be present. The presence of bigger polygonal cells with vesicular nuclei, prominent nucleoli and more obvious eosinophilic or amphophilic cytoplasm indicates ganglion cell differentiation (=ganglioneuroblastoma). On immunohistochemitry, neurofilament expression is usually seen, as well as synaptophysin or chromogranin. There is no expression of CD99, keratins or muscle markers. The MYCN oncoprotein is amplified in +/- 25% of cases and its amplification is associated with advanced stage tumors and an adverse outcome. 1p deletion is also found, which is prognostically also adverse (10-12).

Small cell synovial sarcoma

Synovial sarcoma (SS) is also a misnomer, nobody knows the origin of this tumor but everybody agrees that it has nothing to do with synovium. It is typically a tumor of young adults, nearly half the cases arise in patients younger than 30 years. SS can occur in virtually any anatomic location, the most common place being the lower limb, followed by the upper limb and head& neck region, notably in the parapharyngeal space. SS is generally an aggressive tumor, with metastasis to lung, bone and lymph nodes in up to 50% of cases. Usually, SS shows a monophasic spindle cell pattern, a biphasic pattern is less frequent. Rarely, SS presents as a small blue round cell tumor, thus strongly resembling ES/PNET. This poorly differentiated variant is more aggressive (13. A number of features help to differentiate this

variant from ES/PNET. Poorly differentiated small cell SS usually shows a hemangiopericytoma like vascular pattern. Keratin or CD99 stains are of no use since both tumors can express these markers. Strong EMA expression favours SS over ES/PNET. TLE1, derived from gene profiling studies, is a sensitive (but not specific) marker for SS. More than 90% of SS have a balanced reciprocal translocation t(X;18)(p11;q11), with fusion of SSX and SYT genes. The SSX gene has 5 subtypes (14).

Round cell liposarcoma

Round cell liposarcoma (LPS) represents the high grade end of the spectrum of myxoid/round cell liposarcoma. Fortunately, pure round cell morphology is rare, but, if present, it represents quite a diagnostic challenge. Myxoid/round cell LPS is the second most frequent type of liposarcoma and has a peak incidence in the 4th and 5th decade. It represents the most frequent liposarcoma type in patients younger than 20 years old. The deep soft tissue of the thigh is the most frequent location. The metastatic pattern (very rare in pure myxoid types) is unusual, in that they often spread to unusual places like the retroperitoneum (15). In pure round cell LPS, the myxoid matrix is overrun by closely packed tumor cells with round hyperchromatic nuclei, which often obscure the typical plexiform vascular pattern. Lipoblasts are usually not present, but should be looked for. There is no diagnostic immunohistochemical marker for round cell LPS, and to confuse things more, they can be CD99 positive. A diagnosis of pure round cell LPS should rely on the finding of the typical t(12;16)(q13;p11) which is present in 90% of myxoid/round cell LPS, no matter what the proportion of myxoid/round cell areas is. The translocation leads to a FUS-CHOP fusion. Rare cases show a t(12;22)(q13;q12) as variant translocation, leading to a EWSR1-CHOP fusion (16).

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INVITED LECTURES – BELGIUM

L 27

The story of the Cancer Registry, part I: from cancer incidence registration to a virtual tumour bank

L 28

The story of the Cancer Registry, part II: the Belgian Virtual Tumour Bank, a tool for translational cancer research

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Translational medicine and the need for large scale biobanks

Cancer is one of the leading causes of death worldwide. In Belgium 59,996 patients were diagnosed with cancer in 2008 (1). This number is increasing due to the ageing population, increasing the need for more efficient preventive strategies and therapies. Therapies are increasingly focused on highly specific disease entities, correlating with a wide range of different molecular pathways (2). To identify these pathways there is a need for highly accurate biomarkers. Over the last decades, tremendous biological and technological advances have been observed. However these insights were only limitedly translated into clinical practice (3). Translational medicine focuses on bridging this gap by focusing on direct diagnostic or therapeutic applications of basic research findings (4). One of the essential tools of translational medicine is the availability of high quality human samples and hence well-organized biobanks (5). Biobanks are organized collections of (human) biological material - and associated information - stored for research purposes. Tumourbanks are disease-oriented biobanks, which store residual (left-over) tumour samples and are mostly located in pathology departments. Although the availability of tumour samples in the biobank facilitates translational cancer research, the number of available samples within research institutions is often limited, especially for rare disease entities or specific subtypes of interest. To overcome this problem, central collections are necessary. This can be performed by a central physical collection entity or by a common online database (a virtual collection). The Belgian Virtual Tumourbank is an example of the latter. The aim of this initiative is to provide one central database where the data of all the available samples of the major academic tumourbanks in Belgium can be consulted by researchers, allowing them to trace the samples to the involved local biobanks.

The national cancer plan and the virtual tumourbank initiative

The first initiative towards the creation of a Belgian tumourbank network was taken in 2007, gathering together oncologists and pathologists from five university hospitals (i.e. UZ Leuven, UZ Gent, Cliniques universitaires Saint-Luc, Hôpital Erasme and Institut Jules Bordet). This first consortium evaluated the biobanking situation in the participating

institutions, adopted the model of a virtual biobank, to be managed by the Belgian Cancer Registry (BCR), and set objectives in order to extend this Biobank project in 2008 to all the major Belgian university hospitals (UZ Antwerpen, UZ Brussel and CHU de Liège joined the network). In March 2008 the Belgian National Cancer Plan (NCP) was launched by Mrs. L. Onkelinx, Federal Minister of Social affairs and Public Health. To promote translational cancer research and the collaboration between different cancer researchers in Belgium, one of the initiatives of the NCP (initiative 27) was the creation of a Belgian virtual tumourbank. For this purpose a total budget of 3 million euro was released (6). The criteria for recognition were stated in the royal decree of September 20th 2009 (7). Currently 11 hospitals, among which all major Belgian university hospitals, are recognized and financed by this initiative (see figure 1) . These hospitals all have a local biobank, which stores human residual material (including tumour samples), together with relevant clinical data.

Figure 1 The partners of the Belgian Virtual Tumourbank network

The members of the steering committee of the Belgian tumourbank are representative physicians of all 11 hospitals involved (including pathologists or hematologists, responsible for the activities of the local biobanks; radiation oncologists and medical oncologists), the BCR, the Federal Ministry of Health and the Belgian Cancer Center. The BCR coordinates these activities and is responsible for the set-up of the central database, including all IT needs that are implied.

The dataset of the Belgian virtual tumourbank

One of the first challenges of this virtual tumourbank was the creation of a minimal dataset, i.e. a minimal set of clinical and technical data necessary for researchers to perform a reliable query in the catalogue of the tumourbank. Table 1 gives an overview of the variables that are currently present in this dataset. Besides clinical and technical data, the tumourbank will also contain the national registry number (evidently not consultable for researchers). One of the strong assets of the use of the national registry number as a unique patient identification number by a third party (the BCR) is the possibility to link the tumourbank data with all kinds of relevant clinical and longitudinal data. This linkage not only allows performing the necessary quality controls and adding extra clinical information but also creates the possibility to retrieve extra information of potential scientific interest (e.g. data on patient vital state), even years after the sample is taken from the donor. The use of this minimal dataset together with the national registry number was approved by the Belgian Privacy Commission in 2011 (8, 9).

Table 1 BVT minimal dataset

Local biobank
Creation date
Catalogue ID
National Registry N° *
Gender
Birth date *
Age
Sample ID
Biopsy number *
Sample date
Conservation mode
Conservation delay
Origin of the sample
Available materials
Sample type
Sample localization (ICD-O)
Laterality
Morphology (ICD-O)
Degree of differentiation
pTNM
cTNM
** Identifiable data, not available for researchers*

The web applications BVTr and BVTc

A web application was developed to satisfy the needs for this virtual tumourbank. This application is secured by the User and Access Management of the eHealth

platform. This system performs an eID-based identification and authentication of the user, before the application can be accessed. The application consists of 2 modules: the Belgian virtual tumourbank registration and catalogue module (BVTr and BVTc) (see figure 2). The **BVTr** is used by both the local biobanks and the BCR. This module allows local biobanks to register samples in a central tumourbank database, hosted by the BCR. Registrations can be performed both on a sample by sample basis and by batch upload, depending on the system used in the local biobank. Furthermore the application provides the possibility to update, delete or download the registered sample data. When samples are registered in the central database, the BCR validates these data and publishes them in the (de-identified) database of the Belgian virtual tumourbank. When sample data are not valid they are rejected and automatically sent back to the local biobank for correction. Furthermore the BCR links this central database to the cancer registration database. This linkage allows quality control on the data (e.g. verification whether tumour topology and morphology are matched) and the addition of missing values (e.g. cTNM). Furthermore when extra information, apart from the available minimal dataset, is needed, a researcher can request and retrieve this information, assuming that the involved research project and request is approved by the Privacy Commission. The **BVTc** is a query application for researchers, allowing an easy-to-use consultation of the de-identified database of the BVT. In the near future this application will also offer the possibility to order samples at the local biobanks directly, to reduce the administrative burden of sample requests to an absolute minimum. The BVT web applications are accessible for local biobanks since the beginning of 2012. As most of these biobanks are operational for several years already, the BCR is completing the existing, "historical" data (which are often incomplete because the minimal dataset was not determined yet). Based on these "historical" data, it is estimated that at least 15,000 samples from individual cancer cases can be put in the virtual tumourbank. Furthermore, based on the yearly numbers of conserved samples in the local biobanks, it is expected that every biobank stores an average number of 200-300 new oncological samples a year, meaning about 3000 new samples a year for the virtual tumourbank (always from individual cancer cases). Furthermore it is expected that this number will increase for future data as a consequence of the impulse of this initiative.

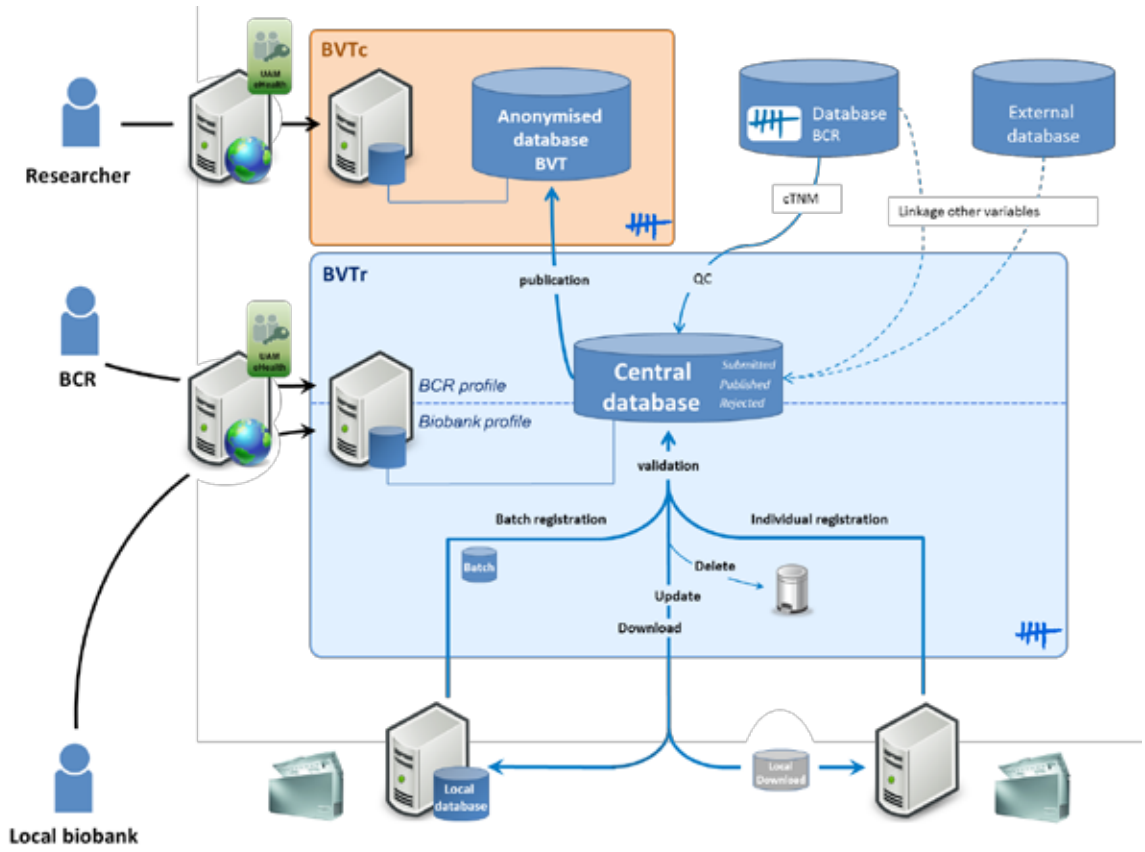

Figure 2 Concept and dataflow in the Belgian virtual tumourbank

An example from translational research

The following case demonstrates the possibilities of the BVT. Suppose a researcher is interested in a potential biomarker of which he hypothesizes that it may be able to predict the response to some kind of existing therapy. To confirm this hypothesis he needs three kinds of information: information on the biomarker (and hence samples to investigate), information on prognosis (survival) and treatment information.
When this research question has to be answered in a prospective study design, this would probably require a multi-center study, taking several years to collect enough sample material and to have reliable follow-up data to calculate survival. The Belgian virtual tumourbank allows a much faster and easier way for this research setting.
The researcher in this case can consult the catalogue of the virtual tumourbank via the BVTc, based on specific search criteria (e.g. age, gender, tumour type, …). Once he has made his selection he can easily send a request to use these samples for his experiments to the local biobanks that store these samples. To achieve data on the vital state and hence survival of the study population a request can be made at the BCR, who can provide this information after approval by the Privacy Commission.
The same procedure can be used for treatment information, which is available in other

external databases. This information allows the researcher to answer his scientific question in a few months, rather than years, as required in a prospective setting.

More information

More information on the Belgian virtual tumourbank can be found on the website, available via www.virtualtumourbank.be. This website provides information about the initiative for both professionals (physicians or researchers) and the broad public, including cancer patients. Apart from the national virtual tumourbank all local biobanks are presented on the website.

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L 32

Simplified approach of a molecular classification of glioblastoma

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Glioblastomas (GBM) are the most common malignant primary brain tumor in adults. Due to their invasive nature and to their poor response to standard treatment, less than half of the patients with GBM survive more than one year. GBM are considered by the World Health Organization classification as a single histologic entity. However, considerable variability in biologic behavior still exits within this entity, resulting in significant differences in terms of prognosis and response to treatment (1). In an attempt to better understand GBM biology, many groups have performed high-scale profiling studies based on gene or protein expression (2-5). These studies have revealed the existence of several subtypes within the GBM entity. While no clear consensus exists, two to four major subtypes seem to emerge from these different studies (6). Interestingly, these different subtypes are associated with different prognoses and present different response to therapy (3, 5). Recently, The Cancer Genome Atlas (TCGA) Research Network has generated a comprehensive catalog of genomic abnormalities in a large cohort of GBM (7). Verhaak et *al*. have used TCGA data to correlate gene expression-based GBM subtypes with alterations in DNA sequence and copy number. They have thereby established a classification of GBM into Classical, Mesenchymal, Proneural and Neural subtypes. The three first subtypes were characterized by genomic abnormalities of Epidermal Growth Factor Receptor (EGFR), Neurofibromin 1 (NF1), Platelet Derived Growth Factor A (PDGFRA), Isocitrate Deshydrogenase 1 (IDH1) and p53 (5).
In the present study, we investigated an alternative approach based on immunohistochemistry to achieve such a molecular classification. For this purpose, EGFR, PDGFRA and p53 immunohistochemistry were evaluated on a retrospective cohort of 100 GBM surgical samples. The quantification of these immunostainings using the Vysiomorph software package (Visiopharm) allowed us to identifiy two GBM subtypes based on the classification established by Verhaak et al. (5). One subtype, named “Classical-like” (CL), is characterized by EGFR positive staining and the absence of both p53 and PDGFRA staining. The other subtype, named

"Proneural-like" (PNL), is characterized by p53 and/or PDGFRA positive staining. This classification is an independent prognostic factor in terms of overall survival ($p = 0.011$) as compared to age, extent of surgery and adjuvant treatment. The patients belonging to the PNL subtype present a significantly longer survival than the patients belonging to the CL subtype (overall survival median of 10.5 vs 5.0 months; $p = 0.047$). Moreover these two subtypes of GBM show different responses to chemotherapy. The addition of temozolomide to radiotherapy significantly improves survival for patients belonging to the CL subtype (overall survival median of 13.4 vs 4.3 months; $p = 0.005$) but it doesn't affect survival for patients belonging to the PNL subtype (overall survival median of 13.5 vs 11.7 months; $p = 0.25$).
This study thus shows that quantitative immunohistochemistry involving only three biomarkers makes possible the identification of clinically relevant subtypes of GBM. This approach has the advantages to be easily applicable on daily practice and to allow large scale-clinical application.

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L 34

Chemokines in colorectal cancer

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Chemotactic cytokines, designated chemokines, have been discovered as essential mediators of directional migration of leukocytes, also called chemotaxis. These small proteins (about 10 kDa) are secreted by a variety of cell types such as leukocytes, epithelial cells, fibroblasts, endothelial cells and tumor cells, either constitutively or after induction by inflammatory stimuli [1;2]. Chemokines play a role during physiological processes by mediating lymphoid tissue organogenesis and lymphocyte homing. In addition, chemokines are also involved in pathological processes including auto-immune diseases, e.g. inflammatory bowel diseases (IBD), as well as tumor growth and metastasis [1].
Chemokines have been subdivided in four families based on the relative position of their cysteine residues located in the N-terminal region: CXC, CC, C and CX_3C chemokines, in which the X represents any other amino acid [1;2]. Chemokines mediate their functions through binding to seven transmembrane G-protein-coupled receptors defined as CXCR, CCR, CR or CX_3CR. Furthermore, there is a high degree of redundancy since some chemokines activate multiple receptors and some receptors recognize more than one chemokine. In addition to interacting with signaling and non-signaling seven transmembrane chemokine receptors, chemokines also bind to glycosaminoglycans (GAGs) [3]. Chemokines modulate tumor behavior by three important mechanisms: regulation of angiogenesis, activation of a tumor-specific immune response and direct stimulation of tumor proliferation in an autocrine or paracrine fashion [4].
Chemokines have been implicated in both processes of vascular growth (angiogenesis and vasculogenesis) and may exert their regulatory activity directly or indirectly as a consequence of leukocyte infiltration. Angiogenic ELR^+ CXC chemokines include growth-regulated oncogene-α (GRO-α)/CXCL1, GRO-β/CXCL2, GRO-γ/CXCL3, epithelial cell-derived neutrophil activating peptide-78 (ENA-78)/CXCL5, granulocyte chemotactic protein-2 (GCP-2)/CXCL6, interleukin-8 (IL-8)/CXCL8 and neutrophil-activating peptide-2 (NAP-2)/CXCL7 [4]. SDF-1 lacks the ELR-motif, but nevertheless has angiogenic properties [4]. In addition, SDF-1 mediates the process of vasculogenesis by attraction of endothelial progenitor cells [4]. CXCR3 binding ELR^- CXC chemokines have angiostatic activities and include platelet factor-4 (PF-4)/CXCL4, platelet factor-4 variant (PF-4var)/CXCL4L1 [5], monokine induced by interferon-γ (Mig)/CXCL9, interferon-γ inducible protein-10 (IP-10)/CXCL10 and

interferon-inducible T cell alpha chemoattractant (I-TAC)/CXCL11 [4].
A causal link between chronic inflammation and cancer has been demonstrated and in the gastrointestinal tract, many chronically inflamed organs are prone to develop tumors. For example, compared to the general population, patients with inflammatory bowel disease (IBD) have a two-to-three fold greater lifetime risk of developing colorectal cancer. In general, the balance between the infiltrated pro-tumoral and anti-tumoral leukocytes determines the outcome of tumor immunity. The chemoattraction of either pro-tumoral or anti-tumoral leukocytes depends on the secretion of ELR^+ and ELR^- CXC chemokines, respectively. During early stages of carcinogenesis, innate responses in which granulocytes, macrophages and NK cells initiate tumor rejection, are likely involved in the activation of effective immune surveillance requiring action of adaptive immune cells such as DC and lymphocytes [6].
Monocytes attracted by chemokines, especially CC chemokines, produced by tumor cells and stromal cells may be the first line of defense in tumors as they colonize rapidly and secrete cytokines and chemokines, which attract and activate immature DC and NK cells [7;8]. Killing of tumor cells by M1 macrophages and NK cells stimulates the release of tumor antigens which are captured and processed by DC and M1 macrophages. Type I $CD4^+$ T cells (Th1), producing IFN-γ, facilitate tumor rejection by providing help to cytotoxic $CD8^+$ T cells (CTL), whereas type II $CD4^+$ T cells (Th2), which secrete IL-4, IL-10 and IL-13, facilitate antibody production by B cells [9]. These tumor-reactive monoclonal antibodies are believed to have anti-tumor efficacy [9]. Besides differentiating into Th1, Th2 and CTL, naïve T lymphocytes can also differentiate into $CD4^+$ T regulatory cells (T_{reg}) and $CD4^+$ Th17 cells. In particular, T_{reg} cells allow tumor development by blocking the activation of CTL and the killing capacity of NK cells [10]. On the contrary, it is not yet clear whether Th17 cells promote or inhibit tumor progression [11].
Even though an adaptive immune response can be provoked during tumor progression by antigen-specific T lymphocytes, such anti-tumor immune response generates immune selection pressure and only tumor cell variants adapted to escape the immune response are able to persist [6]. Furthermore, tumor-derived soluble factors such as cytokines and chemokines (e.g. ELR^+ CXC and CC chemokines) may facilitate the escape from immune attack by altering the immune response in favor of pro-tumoral leukocytes. Indeed, the differentiation of monocytes into type II (M2) macrophages in response to IL-4, transforming growth factor-β (TGF-β), IL-10 or IL-13 stimulates tumor growth by the release of cytokines (e.g. VEGF), chemokines (e.g. IL-8, SDF-1) and matrix-degrading enzymes which are responsible for tuning inflammatory responses, stimulating tumor cell proliferation and promoting angiogenesis [12].
In addition to macrophages, mast cells and neutrophilic granulocytes can also support tumor progression. Neutrophils, which are attracted by ELR^+ CXC chemokines such as IL-8, ENA-78 and GCP-2, exhibit a dual role in tumor development [13]. At one hand, neutrophils can contribute to immune surveillance as they have potent cytotoxic ability and interact with the adaptive immune system. On the other

hand, neutrophils can favor malignancy by releasing growth stimulating signals, matrix-degrading proteases (e.g. matrix-metalloprotease-9/gelatinase B), as well as mediators of angiogenesis such as IL-8 and VEGF [13]. Recently, Fridlender *et al.* have demonstrated that tumor-associated neutrophils possess anti-tumoral (N1) or pro-tumoral (N2) activities depending on their phenotype, which is regulated by TGF-β [14]. Tumors lacking the expression of TGF-β favor the accumulation of N1 neutrophils, which possess enhanced expression of immunoactivating cytokines and chemokines and show increased capacity to kill tumor cells. However, many tumors are characterized by the over-expression of TGF-β mediating the differentiation of neutrophils toward the N2 pro-tumorigenic phenotype [14].

In conclusion, the main pathological feature of IBD is a profound infiltration of neutrophils, lymphocytes, and monocytes in the inflamed tissues of the intestine. These cells are attracted by pro-inflammatory chemokines of which elevated levels strongly correlate with the grade of disease. The inflammatory microenvironment in IBD is tumor-supporting and further results in the progression of IBD promoting tumor initiation, progression, angiogenesis, and metastasis. Other chemokines (ELR$^-$ CXC chemokines) suppress intestinal tumor growth through promoting antitumor immunity [15;16]. In addition, chemokines directly target chemokine receptors expressed on epithelial cells, potentially inducing epithelial cell transformation, proliferation, and migration/invasion. Thus, it would be interesting to evaluate whether neutralizing antibodies or antagonists of pro-tumoral chemokines or alternatively agonists of tumor suppressing chemokine receptors, can serve as drug targets for prevention and treatment of IBD and colorectal cancer.

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L 37

Helicobacter suis, the other *Helicobacter*

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Already in the early days of *Helicobacter* research, pathologists identified patients where they did not find the typical comma-shaped *Helicobacter (H.) pylori* organisms in their histological sections, but rather bacteria with a typical large spiral-shaped morphology. In literature, these bacteria are often referred to as "*Helicobacter heilmannii*". Recent investigations, however, have revealed that this group of non-*H. pylori* helicobacters (NHPH) comprises different species which have been detected in the stomach of different animal species (Haesebrouck et al., 2009). Therefore, at present, the use of the name "*H. heilmannii*" should be restricted to the species as described by Smet and coworkers (2012). Alternatively, the terms *H. heilmannii* sensu lato (s.l.) and *H. heilmannii* sensu stricto (s.s.) may be used to refer to the whole group of non-*H. pylori* helicobacters and to the specific species, respectively (Haesebrouck et al., 2011).

The most common gastric non-*H. pylori Helicobacter* species in humans is *Helicobacter suis*, naturally occurring in the stomach of the majority of pigs worldwide. It has been shown that living in close proximity to dogs, cats and especially pigs is a significant risk factor for contracting a NHPH infection (Meining et al., 1998). Besides contact with animals, we recently showed that *H. suis* may be present on and can survive in pork meat, suggesting that consumption of pork meat may also serve as a source of infection for humans.

In pigs, i.e. the natural host for this bacterium, *H. suis* infection has been associated with ulcers of the non-glandular pars oesophagea of the stomach, although *H. suis* bacteria probably do not colonize this specific stomach region (Hellemans et al., 2007). In recent experimental infection studies, we were not always able to confirm this, although severe ulcerative lesions are consistently observed more frequently in *H. suis*-infected animals, compared to uninfected control animals. Mainly in the antrum, a clear correlation is observed between *H. suis* infection and an increased diffuse infiltration with lymphocytes and plasma cells, as well as the presence of a higher number of lymphoid follicles in the superficial and deep mucosa. In addition, *H. suis*-infected animals show a marked decrease of the daily weight gain. The mechanism underlying this finding remains to be investigated.

Reports in literature about human NHPH infections often lack a detailed description of the exact causative *Helicobacter* species and should therefore be interpreted with caution. In general, clinical symptoms in NHPH-infected individuals include acute or chronic epigastric pain, nausea, dyspepsia, heartburn, vomiting,

hematemesis, abdominal pain, irregular defecation frequency and consistency, and dysphagia, often accompanied by a decreased appetite. Microscopic examination of gastric biopsies of non-*H. pylori Helicobacter*-infected patients often reveals a chronic gastritis, characterized by infiltration with lymphocytes and plasma cells, sometimes organized into lymphoid aggregates or follicles (Morgner et al., 1995; Stolte et al., 1997; Sykora et al., 2003; Van Loon et al., 2003). Compared to the typical *H. pylori*-associated gastritis, gastritis associated with non-*H. pylori* helicobacters is often most pronounced in the antrum, mostly less active and less severe (Stolte et al., 1997). Less frequently, it is complicated by other pathologies such as atrophic gastritis and intestinal metaplasia (Debongnie et al., 1995; Stolte et al., 1997), although it can be accompanied by ulcerations (Debongnie et al., 1998), mostly in the antrum, and even by gastric MALT lymphoma. Interestingly, the risk of developing MALT lymphoma seems higher with non-*H. pylori* helicobacters than with *H. pylori* (Stolte et al., 1997 and 2002; Morgner et al., 2000).

Histopathological changes described in infected humans were confirmed in Mongolian gerbils and mice infected experimentally with *H. suis* (Flahou et al., 2010). Most infected gerbils showed a marked lymphocytic infiltration in the antrum. From 9 weeks after infection onwards, lymphocytic aggregates contained a majority of CD20-positive B cells, mostly organized in germinal center-presenting lymphoid follicles. In gerbils infected for 8 months, these germinal centers were often large, hyperproliferative and irregular. Additionally, severe destruction of the normal antral architecture at the inflamed sites and development of mucosa-associated lymphoid tissue (MALT) lymphoma-like lesions, such as lymphoepithelial lesions and infiltration of the tunica muscularis, were observed in some gerbils. In part, this different pathology compared to *H. pylori*, could be explained by the evoked immune response, which has been shown to be mainly T helper (Th)17/Th2 polarized, which is clearly distinct from the Th17/Th1-directed immune response seen with a *H. pylori* infection.

In addition to the changes related to *H.* suis-induced gastritis, an association has also been shown between *H. suis* colonization and necrosis of parietal cells, both in mice and Mongolian gerbils, mainly at the transition zone between corpus and antrum. Recent *in vitro* studies have revealed that *H. suis* possesses an important virulence factor, γ-glutamyl transpeptidase, involved in the induction of gastric epithelial cell death. The enzyme catalyzes cleavage of extracellular glutathione into pro-oxidant glutathione degradation products, which bring on cell damage and cause apoptosis or oncosis, depending on the amount of extracellular glutathione available as a GGT substrate (Flahou et al., 2011).

Further studies are currently ongoing to unravel the typical host immune response evoked by *H. suis* and to determine which virulence factors contribute to the whole repertory of gastric changes induced by *H. suis* infection, both in pigs and humans.

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P-glycoprotein in lymphoplasmacytic enteritis in dogs, a model for Crohn□s disease in humans?!

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Crohn's disease

Crohn's disease (CD) belongs to the 'inflammatory bowel diseases' (IBD) in humans, which represents a group of idiopathic, chronic, inflammatory intestinal conditions. Its 2 main disease categories are Crohn's disease (CD) and ulcerative colitis (UC), with both overlapping and distinct clinical and pathological features. CD is typically a disease of ileum and colon, but can also affect other areas of the digestive tract and involves the whole intestinal wall (Stange et al., 2006). UC is an ulcerative and inflammatory disease usually limited to superficial layers of the colon (Stange et al., 2008)

The pathogenesis of IBD in humans is incompletely understood. Genetic and environmental factors such as altered luminal bacteria and enhanced intestinal permeability play a role in the dysregulation of intestinal immunity, leading to gastrointestinal injury (Bernstein et al., 2010).
The intestinal integrity is maintained by several factors including the epithelial cell layer, mucus-secreting goblet cells, antimicrobial peptides-producing Paneth cells, IgA-releasing plasma cells, and gut-associated lymphoid tissue such as Peyer's Patches (Artis, 2008; Barnes et al., 2009). Current theories on the pathogenesis of CD suggest that the intestinal inflammation is due to an abnormal and prolonged Tcell-mediated immune response directed against the commensal gut microbiota that occurs in genetically susceptible individuals after, as yet undefined environmental insults (Packey et al., 2009).
According to a recent epidemiological study by Molodecky et al. (2012), the incidence and prevalence of IBD are increasing with time and in different regions around the world, indicating its emergence as a global disease.

LPE in Dogs

IBD is well known in humans; however, these disorders are also present and investigated in animals. IBD in small animals is defined as an idiopathic chronic inflammation of the stomach, small and/or large intestine (Day *et al.*, 2008; Washabau *et al.*, 2010). In dogs, lymphoplasmacytic enteritis (LPE) is one of the most important chronic diseases of the small intestine that is included within the spectrum of IBD (Cerquetella et al., 2010).

The pathogenesis of LPE in dogs is poorly understood. Experimental models suggest

that disruption of one of three critical areas: the mucosal immune system, the endogenous microflora or the mucosal barrier, results in chronic inflammation (German *et al.*, 2003). Thus far, most studies of canine LPE have focused on the mucosal immune system and the intestinal microflora. In particular, the composition of the microbiota (Xenoulis et al., 2008) and the role of IgE (Locher et al., 2001), lamina propria lymphocytes (Allenspach et al., 2006a), lymphocyte apoptosis (Dandrieux et al., 2008), Toll-like receptors (Burgener et al., 2008), NF-κB (Luckschander et al., 2010) and cytokines (German et al., 2000; Peters et al., 2005; Jergens et al., 2009) have been investigated. Factors involving mucosal barrier disruption have not been investigated extensively in dogs with LPE. Changes in permeability of the intestinal epithelium may have consequences for antigen and drug movement across this barrier. Indeed, Sorensen *et al.* (1997) and Kobayashi *et al.* (2007) both reported a change in mucosal permeability in dogs with LPE, though these findings were in conflict with the results of Allenspach *et al.* (2006b).

P-glycoprotein

P-glycoprotein (P-gp), the product of the multidrug resistance gene *MDR1*, is a membrane-bound efflux pump, highly expressed in tissues with a barrier function such as the blood-brain barrier and the intestinal surface (Thiebaut *et al.*, 1987; Cordon-Cardo *et al.*, 1989). P-gp is involved in the transport of a wide range of small molecules. Epithelial P-gp expression may have a role in protection from bacterial products and xenobiotics in the intestine and also may affect the bioavailability of drugs used in the treatment of LPE (Ho *et al.*, 2005). Therefore, we evaluated the expression of P-gp by the intestinal epithelium of dogs with LPE compared with disease-free animals.

Study on P-glycoprotein expression in LPE dogs

This retrospective study included 57 dogs with a clinical and histopathological diagnosis of LPE. The dogs were of 29 different breeds and ranged in age from 1–13 years. All dogs were presented to the Small Animal Clinic of the University of Ghent with persistent (> 3 weeks duration) gastrointestinal signs. Clinical investigation included complete blood count and serum biochemistry and dogs were treated with parasiticides and antibiotics. Other possible causes of chronic diarrhoea were excluded. Animals were assigned a clinical score by use of the canine chronic enteropathy clinical activity index (CCECAI) as proposed by Allenspach *et al.* (2007). This clinical scoring system includes all parameters of the canine inflammatory bowel disease activity index developed by Jergens *et al.* (2003) (i.e. attitude/activity, appetite, vomiting, stool consistency, stool frequency and weight loss), but also includes assessment of serum albumin concentration and the presence of ascites, peripheral oedema and pruritus. Forty four of the 57 dogs had not been treated with glucocorticoids before biopsy samples were taken, while 13/57 had received glucocorticotherapy at the time of biopsy.

Endoscopic or full-thickness biopsies of the duodenum were available from most animals. Samples of the jejunum, ileum and colon were available from only a few dogs. Endoscopic biopsy samples from the duodenum and colon of 16 healthy beagles (2–6 years of age) were used as control tissues. Collection of the control samples was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University (EC 2007/073).

Biopsy samples were fixed in neutral-buffered formalin, embedded in paraffin wax, sectioned (4µm) and stained with haematoxylin and eosin (HE). All duodenal biopsies were evaluated according to the histopathological standards of the WSAVA Gastrointestinal Standardization Group (Day *et al.*, 2008). These standards include assessment of morphological features (i.e. villus stunting, epithelial injury, crypt distension, lacteal dilation and mucosal fibrosis) and inflammation (i.e. intraepithelial lymphocytes and lamina propria lymphocytes, plasma cells, eosinophils and neutrophils). As proposed by Day *et al.* (2008), numerical addition of grades of histopathological change (0, normal; 1, mild; 2, moderate; and 3, marked) provided an overall histological score for each biopsy sample.

The monoclonal antibody C219 (SIG-38710; SA Eurogentec, Ougreé Seraing, Belgium) was applied to sections taken from the wax blocks at dilution of 1 in 10.
The immunohistochemistry (IHC) was performed in an automated immunostainer (Dako, Glostrup, Denmark). For 'visualization' the Envision/HRP mouse (DAB+) kit (DK-2600; Dako) was used.
P-gp immunolabelling was scored using a semiquantitative scoring system: 0, no epithelial P-gp expression; 1, P-gp expression by epithelial cells at the level of the villus tips in the small intestine or multifocal loss of P-gp expression at the plasma membrane of colonic enterocytes; and 2, continuous P-gp labelling at the brush border of the surface epithelium.

Epithelial P-gp expression was induced in the small intestine in 20 of the 57 dogs with LPE, while in the control dogs there was no detectable P-gp expression in the duodenal epithelium. A minority of dogs with LPE had partial loss of P-gp expression in the colonic surface epithelium.
The reason for this is unclear; however, possible explanations may be proposed based on experimental studies:
Mutant mice lacking P-gp expression (*MDR1* knock-out) develop spontaneous colitis in a specific pathogen-free environment (Panwala *et al.*, 1998; Banner *et al.*, 2004). This suggests that loss of P-gp expression may lead to intestinal inflammation. Similarly, Blokzijl *et al.* (2007) reported a decrease of P-gp mRNA expression in inflamed tissue from patients with Crohn's disease, ulcerative colitis, collagenous colitis and diverticulitis. However, in contrast to these findings, Buyse *et al.* (2005) described a significant increase in P-gp expression in the ileum and reduced P-gp expression in the

colon in rats with experimentally-induced colitis. Dextran sulphate-induced colitis in mice was also characterized by reduced colonic epithelial expression of P-gp (Lizasa *et al.*, 2003). These experimental data suggest that changes in P-gp expression may be a consequence of inflammation.
Another explanation for elevation in P-gp expression has been hypothesized by Fakhoury *et al.* (2006). These authors reported more mRNA encoding P-gp in the duodenum of children with Crohn's disease and suggested that this increase may represent an adaptive mechanism, compensating for reduced P-gp expression and activity in the colon. Unfortunately, it was not possible to explore this hypothesis in the present study due to the lack of colonic biopsy samples from animals with P-gp induction in the small intestine. Relatively more ileal (8/11) than duodenal biopsy samples (14/54) from LPE dogs had P-gp expression. It is known that histopathological changes are sometimes more readily detected in the ileum than the duodenum of dogs with IBD (Casamian-Sorrosal *et al.*, 2010). It is possible that changes in P-gp expression also are more readily detected in ileal than duodenal samples, but unfortunately we lack samples from the ileum of the control dogs.
No association has been found so far between the severity of histological changes and long-term outcome or prognosis in canine IBD (Münster *et al.*, 2006; Allenspach *et al.*, 2007; Garcia-Sancho *et al.*, 2007). Further follow-up studies are necessary to investigate the correlation between P-gp expression and long term prognosis.

Based on the findings of this study, it may be concluded that intestinal P-gp expression is increased in the small intestine of many dogs with LPE and decreased in the colon of some LPE patients. It is not clear whether this is a cause or a consequence of the inflammation. There was no correlation with clinical score, histological severity or previous glucocorticoid treatment. This change in P-gp expression in dogs with LPE may affect the permeability of the mucosal barrier and may have consequences for treatment response, as P-gp limits the absorption of substrate drugs by transporting them from the enterocytes into the gut lumen (Lin and Yamazaki, 2003). In this way, the bioavailability of P-gp substrates such as the immunosuppressant drugs used in the management of IBD may be affected, and this may have consequences for treatment efficacy.

Conclusion

Although some striking different forms of IBD exist in humans and dogs (Cerquetella et al., 2010), the study of IBD in both humans and dogs has led to the hypothesis that genetic factors and enteric bacteria can play a pivotal role in the pathogenesis and response to therapy of this disorder.
The role of P-gp in IBD is still controversial in both man and dog and the existing animal models are mainly based on genetically engineered rodents. The parallel study of IBD in dogs, as performed in this study, might lead to important information in man on genetics, microflora and therapy efficacy. Therefore, we would like to encourage further research on LPE in the dog.

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Limitations in thyroid fine needle aspiration biopsy

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The assessment of thyroid nodules is a common clinical problem. The frequency of thyroid .nodules detected byultrasound (US) has strongly increased in recent years, reaching 67% of the adult population (1). In contrast, thyroid cancers are rare, accounting for only 1% of all cancers and occurring in w5% of all thyroid nodules independent of their size (1, 2). Thus, the challenge that the physicians who manage patients with thyroidnodules face is the efficient stratification of patients according to their risk of malignancy in order to identify the best follow-up and therapeutic options. Fine-needle aspiration (FNA) has become the pre- dominant method used for the primary diagnosis of benign and malignant thyroid nodules, resulting in the categorisation of patients as operative or non-operative
candidates (1–3). However, FNA has intrinsic limitations in distinguishing between benign and malignant follicular lesions (4). More particularly, evaluation and treatment of patients with follicularproliferation (FP) cytology still remain problematic. As this category is associated with a 20–30% incidence of malignancy, patients with this cytological diagnosis are referred for surgery (3). In addition to the developmentof biomarkers applied to FNA (5, 6), efforts to improve the management of these patients have focused on identifying additional clinical and US data that efficiently predict malignancy (7, 8).

We developed a **grading system for FP FNA diagnosis**. Based on our personal experience, our cytological diagnoses were classified into four major categories as detailed below.

- **Unsatisfactory or non-diagnostic samples**:
  This involved specimens that could not be diagnosed because of poor fixation, poor cell preservation or hypocellularity (i.e. less than eight clusters of well-preserved cells at least on each of two slides).
- **Benign diagnosis**:
  This involved specimens categorised as benign, which included i) colloid nodules with abundant colloid and benign follicular cells, ii) colloid nodules with cystic changes consisting of abundant colloid-containing macrophages and benign follicular cells and
- **Lymphocytic thyroiditis**:
  Presenting lymphocytes, plasmacells and oncocytic follicular cells.
- **Follicular Proliferation (FP) diagnosis**:

This category was subclassified into three grades as follows:

a) **Grade 1 FP**: this is applicable to specimens with variable amounts of colloid, including some sheets of follicular cells presenting with low architectural atypia and without cellular atypia. In these specimens, the pathologist favoured a follicular neoplasm, but a benign lesion could not be excluded.

b) **Grade 2 FP**: this is applicable to hypercellular specimens with scant colloid and sheets of follicular cells and with numerous atypical architectural features. In these specimens, the pathologist favoured a follicular neoplasm.

c) **Grade 3 FP**: this is applicable to specimens with grade 2 FP criteria without a papillary pattern. In addition, these samples presented cytological features suggestive of papillary carcinoma (PC), such as nuclear grooves or ground glass nuclei, but not in sufficient quantity or quality for a definitive diagnosis of the follicular variant of PC.

- **Malignant diagnosis**:

  This involved specimens that had cytological features of malignant neoplasms including PC, medullary carcinoma or anaplastic carcinoma.or Lymphoma and metastasis.

Cytological diagnoses of FP and malignancy were considered to be a surgical indication. Certain patients with benign FNA diagnoses were referred for surgery when their clinical or US follow-up suggested a high suspicion of carcinoma, or when the symptoms were caused by nodules and/or patient anxiety.

In our experience, regarding the surgical indication, the sensitivity and the specificity of FNA were 80.4 and 79.8% respectively for the histological diagnoses of adenoma and cancer. The positive and negative predictive values were 82.3 and 77.7% respectively, with a global accuracy of 80.1%. In agreement with other series (9, 10), our results demonstrated that strictly benign or malignant FNA results (i.e. excluding FP) show a great concordance with benign or malignant histological diagnoses. FNA sensitivity and specificity for the diagnosis of malignancy reached 91.5 and 99.1% respectively. The positive and negative predictive values are 94.9 and 98.4% respectively with a global accuracy of 97.8%. In the literature, the reported incidences of false-positive FNA and false-negative FNA range from 0 to 9% and from 1 to 7% respectively (1, 15, 11–15). As highlighted by Yang et al. (10), the large variations of the reported FNA sensitivity and specificity were attributable mainly to the data analysis methods. Most studies exclude the indeterminate cytological categories (i.e. FP) from the computation. This is the reason why two accuracy analyses were carried out in the present study: one studying the accuracy of surgical indication because FNA had been used by clinicians as a screening test to determine whether surgery is required, and the other studying the accuracy of malignancy detection. The first analysis was carried out for the FNA diagnosis of neoplasm (including FP), and the false-positive and false-

negative rates were 17.7 and 22.3% respectively; the second analysis was carried out for distinguishing between benign and malignant FNA diagnoses (excluding FP), and the false-positive and false-negative rates were 5.1 and 1.6% respectively. To refine the FP FNA diagnosis, we introduced a three-level grading system. This grading system was implemented before the publication of the NCI FNA guidelines (3, 16) (which was introduced in 2007–2008), and was improved over time. By including unsatisfactory, benign and malignant FNA diagnoses, we currently use a six-category system as favoured by the NCI FNA guidelines (unsatisfactory, benign, atypia of undetermined significance, follicular neoplasm, suspicious for malignancy and malignant). However, there are two differences pertaining to the NCI FNA guidelines: i) in grade 1 FP, the pathologist favours a follicular neoplasm with a very low probability of cancer. For clinicians, the choice of close clinical follow-up is justified, and a novel FNA can be performed after 6 or 12 months. In the NCI guidelines, the terminology 'atypia of undetermined significance' is defined as cytological findings not convincingly benign, and is not sufficiently informative to allow the clinicians to make an adequate therapeutic decision. It should be noted that some members of Committee IV suggested that this category should be optional (3); ii) as opposed to the NCI classification, we used the diagnosis of 'suspicious for malignancy' only for the follicular variant of PC (grade 3 FP). Our subclassification of FP presents a gradation in malignancy incidence (i.e. 8, 18 and 46% in our series) similar to the recently reported figures (5–10, 20–30 and 50–75%) (3). The new guidelines refer patients with a nodule diagnosed with an FP level of 2 or 3 for surgery (i.e. follicular neoplasm or suspicious for malignancy), whereas close clinical follow-ups with repeat US-guided FNA are recommended for the FP level 1 diagnosis(i.e. atypia of undetermined significance) (3).

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Accreditation: how does it work?

V. NOTEN

F.O.D. Economie, K.M.O., Middenstand en Energie

Accreditation Service – BELAC

Accreditation: what is it?

What is an accreditation? It is an attestation granted by a third party related to a body for conformity assessment, such as a laboratory, an inspection or a certification body.

This certificate is granted by an accreditation body after a thorough assessment based on internationally recognized requirements; it conveys the formal demonstration of the competence of the organization to carry out specific tasks relating to conformity assessment.

Laboratories, inspection and certification bodies may, through accreditation, provide evidence of their technical abilities, but also of their independence and impartiality.

Accreditation: why?

The economic structures are subject to a dynamic evolution forced by the trade internationalisation. Moreover, products and services must meet regulated requirements which aim at guaranteeing their use in full safety.

It is essential to gain the confidence in the conformity of products and services both of the economic actors and of the authorities in charge of the market control.

The conformity is attested on documents joined to the products and are issued by conformity assessment bodies (laboratories, inspection and certification bodies). A product or service accompanied by a report issued by an accredited body inspires increased confidence as to conformity with the laid down specifications, which facilitates market access. It is obvious that accreditation is an instrument favouring free circulation of products and services. It contributes to eliminate technical barriers, to allow fair competition and to achieve harmonization in trade agreements.

Accreditation in Belgium: how?

In Belgium the accreditation structure is based on the Law of July 20, 1990.

Since August 1, 2006, BELAC is the only Belgian Accreditation Body. It was established by the provisions of the Royal Decree of January 31, 2006 and is placed under the responsibility of the FPS Economy, S.M.E.s, Self-employed and Energy.

BELAC operates according to the international requirements with regard to the management of the accreditation bodies.

Accreditations issued under the BELAC roof are recognized by the Belgian State.

Accreditation at international level: how?

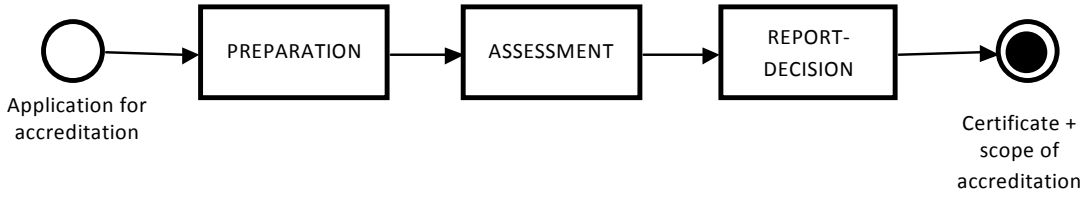
National accreditation bodies are involved in networks at a regional level which, in turn, cooperate on a world wide basis. Mutual recognition of accreditation services is decided only after thorough evaluation through peer review according to strict requirements agreed at international level.

BELAC is a signatory of all existing MLAs (multilateral agreements) and MRAs (multilateral recognition agreements) of EA (European co-operation for Accreditation), ILAC (International Laboratory Accreditation Cooperation) and IAF (International Accreditation Forum).

In this way, reports and certificates issued by BELAC accredited bodies are internationally recognized.

In turn, BELAC recognizes the accreditations delivered by equivalent accreditation bodies with which a mutual recognition is effective and BELAC promotes the acceptance of reports and certificates issued by the accredited bodies.

The accreditation procedure



Submission of application

Each body intending to apply for accreditation and which contacts the BELAC secretariat, will receive documentation providing details on the functioning of BELAC, the procedure and requirements for accreditation, the financial aspects and the way to access the main documents via the homepage of BELAC; this includes the application form.

Composition of the team

The composition of the assessment team, depends on the definitive description of activities for which accreditation is requested.

The composition of the team needs to allow for evaluation of the organisational aspects (lead assessor) and of the technical fields included in the application for accreditation (one or more technical experts).

Assessment visit and report

The evaluation of the way in which the applicant actually works constitutes the most important part of the assessment.

- The lead assessor has the task to evaluate the management system on the basis of the quality manual and documentation .
- The technical assessors and experts have to examine the management system in operation and to assess the ability of personnel to carry out specific tasks. The evaluation includes witnessing of actual activities.

Findings noted by the assessors will be both factual and objective and will refer to the relevant accreditation requirements. Analysis of findings gained during an assessment can lead to identify non-compliances .
At the end of the assessment, each assessor formulates his/her findings and prepares a list of non-compliances. Non-compliances are categorized into major and minor non-compliances.
A major non-compliance, also called type A non-compliance, implies that the system does not meet its own objectives nor those of the accreditation requirements and directly jeopardise the quality of the activities and/or the effectiveness of the management system. A minor non-compliance, also called type B non-compliance, is a non-compliance without an immediate effect on either the overall quality of the activities or the efficiency of the management system.

After the assessment, the laboratory will perform a cause analysis and an analysis of the extent of the non-compliances and define corrective actions for each non-compliance. The corrective actions for type A non-compliances must be implemented before accreditation can be granted.

The team members will write the assessment report. The report is intended to provide adequate record of the evaluation and evidence of compliance and to allow the Board to make a decision about granting the accreditation.

Granting accreditation

The assessment report, including the corrective actions formulated by the laboratory will be submitted for examination by the Accreditation Board, which will make a decision about the granting of the accreditation and the list of activities for which accreditation is granted for. The decision includes the term of validity of the accreditation, i.e. a maximum of 3 years for a first accreditation cycle and the surveillance program that consists of minimum 2 yearly visits for a first accreditation cycle.

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Accreditation: What does it involve?

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I. Introduction

In recent years, more and more laboratories have entered an accreditation process. In Belgium, since the first August 2009, molecular biology diagnostics during the investigational phase of non myeloid and non lymphoid tumors can only be performed in a laboratory that possess the ISO 15189 accreditation, since this is mandatory for the reimbursement of services. The operational phase of the accreditation is based on the implementation of a european standard (NF EN ISO 15189) that specifies the requirements of quality and competencies in clinical laboratories. The Pathology department from the Erasme University Hospital of Brussels is committed to this approach and is accredited since December 8, 2009.

Accreditation of medical laboratories is based on the establishment of a system of quality management associated to the formalization of the technical competencies of the laboratory. A controlled quality management system relies on the definition of the quality policy of the laboratory (the objectives), mastery of quality documents and a strict traceability.

In parralel, technical competencies are assessed through the continuous formation of staff members, formal certification of personnel to the different tasks, clear definition of responsabilities, consistent technical choices and methods validation. This approach is part of a continuous improvement process based on the quality objectives defined during the management reviews, monitoring of the system by conducting internal and external audits, as well as implementation of preventive and corrective actions to ensure an adequate and constructive response to complaints and dysfunctions.

Although the development of a quality management system relies on the commitment of the laboratory top management, it can only be effective if all staff members are involved. Many efforts have been required from the personnel, at each level, to ensure the efficacy of the quality system.

II. The Quality Management System

- **Management commitment** is the basis for establishing the quality system. The management defines the level of performance to reach, the goals, the choice of the standard and the means employed to achieve the objectives. At this point, a Quality Monitoring Cell can be created and a Quality Manager designed. An action plan is established so that everything is done to meet the requirements of the standard.
- **Documentation management** must be clearly described in a procedure that defines the different types of documents, their nomenclature as well as their life cycle (edition, approval, validation, diffusion method, management of the different versions). The

traceability of records must be described. Common difficulties encountered during the drafting and implementation of this procedure concerns the handling of external documents with regard to their revision, archiving and implementation by the staff. Additionally, an important choice concerns the format of the documentation, for instance the use of either paper documents or computer files.

- **Monitoring and adjustment of the system.** The management review allows determining areas for improvement as well as setting up the required corrective actions. Such decisions are based on quality indicators, complaints and dysfunctions met, as well as the analysis of reports of internal and external audits. The organization of surveys allows the laboratory to evaluate the satifaction of prescribing doctors and is an excellent tool for listening to their needs. The records of non-compliance may initially contribute to a poor perception of the quality management system by the personnel who perceives it as a "policeman". The formations and messages conveyed by the Quality Monitoring Cell are therefore important for the good integration of the concepts by the staff members, and facilitates adoption of the system of quality management. It is important to ensure that the quality system remains appropriate and does not become too cumbersome in its daily application (**cf. fig. 1**). Our laboratory decided to invest in a system that allows tracking of samples throughout the diagnostic chain. This system of traceability has been tailored to suit our needs. In addition to allowing full traceability of samples, it is a management tool that will allow in a near future to ease the workload of technicians and qualiticians, while improving the efficacy of the diagnostic chain. However, adaptation of technicians to this new tool is not easy since it involves a complete reorganization of the work method (continuous flow rather then sequential).

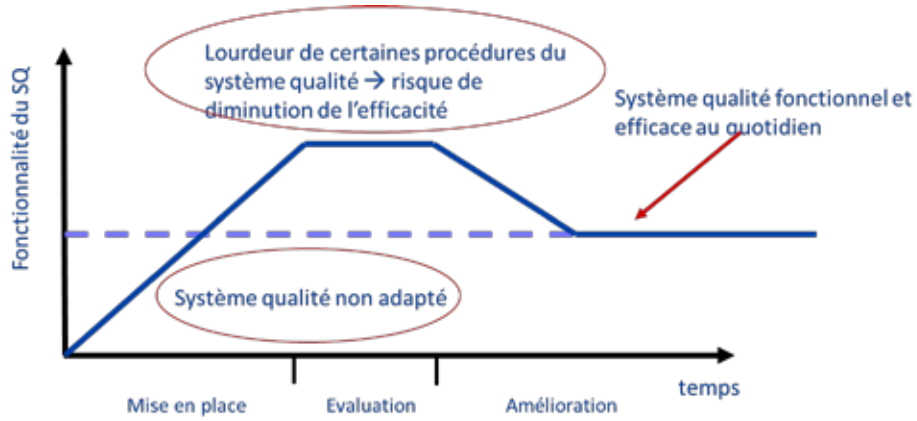

Fig 1 : Evolution of a quality system

III. Technical organization

- **Competencies management** must be ensured by the organization of formations on « quality » and « techniques » that allow each staff member to maintain its skills at the required level, and as defined in its professional profile. Formations must be

evaluated for their effectiveness, with regard to the initial objectives. Staff members must be formally certified before taking office. Difficulties can be met concerning the monitoring of the competencies of external staff or hierarchical superiors (collecting doctors, transporters, surface technicians).

- **Materials and consumables** are managed by a general procedure that details their selection, how to order and store them, as well as their maintenance. Each material and consumable is listed and each activity concerning them is traced. Inventory management of reagents and consumables ensures a continuous supply. Reconstitution of the different solutions is recorded. The lots of reagents and consumables are traced. On the basis of complaints received and the records of failures, suppliers are evaluated. Contracts of reagents and consumables are adapted according to the quantity used, with the purpose of avoiding waste. The raw data/results of each analysis can be immediatly produced if a request is made.
- **Metrological requirements** should be defined as required, depending on the equipments and their criticality. The analytical method for metrological monitoring is developped on the basis of normative or consensual requirements. Practically, a metrological monitoring must be applied to measures of volume (micropipets), temperature (incubators, measuring instruments), weight (scales), time (chronometers) and speed (centrifuges). Metrological monitoring can be performed internally or by an external provider. When implementation of the analytical method is done in-house, care must be taken to ensure that the standard used for the monitoring is connected to the national standard. When the monitoring is performed by en external provider, this provider has to be accredited. Depending on the criticality of the equipment, a system of retrovigilance needs to be established.
- **The pre-analytical phase** is one of the most difficult to monitor, since it also depends on the performance of external services. The laboratory performing the analysis is accountable, which has many implications in terms of quality. Criteria for the acceptance or non-acceptance of samples must be determined and described in a primary samples manual, whose distribution has to be monitored. Every external samples collector must be informed and have an access to the primary samples manual, in its up-to-date version. The laboratory has to ensure that the rejection of a sample is due to a true impossibility to process it. In case of rejection, a challenge is to describe the notion of non-compliance to the prescribing physian. Logistics considerations involve minimizing the time required for the transfer of the sample to the laboratory performing the anlysis.
- **The analytical phase** is easier to monitor. Maximum involvement of qualified personnel has to be ensured. The personnel must have a full documentation for performing the different types of analysis. The methods used must be validated, which is more difficult to achieve in the case of manual methods. Quality controls help ensure the validity of the analysis performed in the laboratory and the correctness of the results

obtained. They are of two types: 1) Internal quality controls that ensure the continuous validation of the techniques. They can be used as indicators, after definition of the procedure to follow when the threshold for technical validation is exceeded; 2) External quality controls must be selected and organized for each analysis, according to the described procedure.

- The post-analytical phase involves the biological validation of the sample by qualified personnel, based on the available clinical data on the patient. A system of monitoring must be defined to ensure that the interpretations harmonized from one reader to another. Validation criteria based on guidelines (when available) or the adequate literature must be described. Storage time and/or destruction of samples after analysis must be clearly formalized. The laboratory has to ensure that the report is sent (and received) within the agreed timeframe by all persons concerned and authorized to receive and use medical informations. The report must be clearly readable and free of transcription errors.

IV. Advantages of establishing a quality management system

The establishment of standardized procedures has improved the quality of our sevices. It has allowed a rationalization of the management and the investments, as well as a harmonization of practices. The permanent monitoring, performed internally as well as externally by BELAC, ensures the everyday efficacy of the system. Personnel management involves clearly defined tasks for which the staff memeber is formally authorized. The personnel must have access to a full documentation that allows acting adequately and addressing the different types of problems that can arise.

V. Difficulties linked to the development of a quality management system

When establishing the system, many efforts have been agreed by staff members at all levels of operation. Approximately two years were required to have the necessary perspective to measure the improvements and provide this feedback. Although tailored and adapted to fit the needs of the laboratory, the system retains some heaviness, especially regarding the validation of techniques since any change must be approved and validated. Considering the specific expenses linked to the accreditation process as well as the daily maintenance of the quality management system, savings are difficult to quantify. External recognition of accredited laboratories is still low, at this date.

VI. Conclusion

The development of a system of quality management is within the reach of most laboratories and is a warranty of high quality for the administrative authorities. The integration of the standard ISO 15189 in the pathological diagnosis allowed us, by the definition of operating rules and the formalization of our technical know-kow, to implement a functional quality system that is efficient on a daily basis. This system has contributed to improving the quality of our services, in terms for instance of efficiency

and traceability. Development and analysis of relevant indicators allowed conducting the appropriate corrective actions, with the purpose of improving our services. The key to success of this approach relies on the full commitment of all staff members to the quality system, their motivation and technical competencies.

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Accreditation: the experience of the clinical biology laboratories.

Prof. J.C. Libeer, former head of the clinical biology department
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Clinical biology is a part of laboratory medicine as defined in ISO documents. According to ISO 15189, medical laboratories are laboratories for the biological, microbiological, immunological, chemical, immunohaematological, haematological, biophysical, cytological, pathological or other examination of materiels derived from the **human** body for the purpose of providing information for the diagnosis, prevention and treatment of disease in, or assessment of the health of human beings, and which may provide a consultant advisory service covering all aspects of laboratory investigation including the interpretation of results and advice on further appropriate investigation. (1)

If we consider the brain to brain loop from Lundberg (2) we observe that in clinical biology laboratories, the aspects preparation, analysis, reporting and interpretation were traditionally well managed. There was much less attention to aspects as ordening, collection, identification and transport. In pathology laboratories also, traditionally quality started only when the sample was put under the microscope. From our personnal experience we saw many laboratories that did not to give clear instructions for sample requirements as they where afraid that to stringent conditions could scare the requester.
Several publications (3-6) have demonstrated that most errors occur in the pre-analytical phase; and this is just the phase that was not well managed until now.
In the whole approach of better quality management, accreditation is not the most important step; it is only the award of recognition that the laboratory conducts a valuable quality management system.
A general accepted worldwide standard for medical laboratories was published by ISO in 2003: ISO 15189. This standard covers the essential elements for medical laboratories to demonstrate the quality and competence of their services, as well as the consistently deliver technically valid test or "examination" results as they are known in the standard. The standard which has been developed with strong involvement from the medical, scientific and clinical community, is for the use of medical laboratories in developing their management systems and maintaining their own competence; and for accreditation bodies to confirm or recognise the competence of these laboratories through accreditation.
In the historical overview of better quality management in laboratory medicine, we saw first CLIA (the clinical laboratory improvement amendments) in the US (7).

CLIA began in the late 1960's when problems arose in the cytology laboratories that read PAP smears. The personnel in these laboratories were overworked and had a very high error rate. Many women suffered or died because the cytologists had missed the early stages of cancer on the PAP smears.
After a hearing in the parliament, the Clinical Laboratory Improvement Amendment was passed in 1967 and the first laboratory regulations were born. In 1988, a second amendment was passed but did not go into effect until 1992 when the new regulations were approved.
The purpose of CLIA was to set minimum standards for all laboratories to follow and to determine if laboratories are achieving those standards.
The Leidschendamse conferences (1989, 1990 and 1995) were at the basis of legislation for patient care (8) in the Netherlands. Several professional organisations published model quality manuals as an example for their members. (9-10)
France published in 1994 the GBEA (guide de la bonne exécution des analyses de biologie médicale) (11). This guide remained in general a dead letter.
As there were concerns on quality the French Assemblée published in 2010 a new law on medical laboratories (12). Formal accreditation becomes mandatory for all medical laboratories from 2016 ab.
In Belgium the first obligation for a quality system in medical laboratories was included in the royal decree of 3 December 1999 (13). Practical guidelines for implementation were explained in a Belgian Guide of Practice (14).
Mandatory accreditation was required in 1996 for the HIV reference laboratories (15), 1998 for the reference laboratory for tropical diseases (16) and in for laboratories performing tests in the nomenclature groups of article 24bis, 33bis and 32 since 2009.
We can see that after a first phase of hesitation, a working quality system was progressively introduced into real live of clinical laboratories. This positive evolution has got a tremendous effect not on the quality of results, but on the outcome quality of these laboratories.
Even for the staff, this evolution was positive and questionnaires confirmed that co-workers feel much more comfortable in the laboratory after the implementation of a quality system.
It is expected, that in a near future, accreditation will become an essential requirement for licensing and reimbursement in al fields of laboratory medicine.

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L 44

Pathology of drug-induced liver disease

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While drug-induced liver injury (DILI) is still infrequent, the incidence is clearly increasing. This is most likely due to the more frequent use of prescription medication in recent decades. For example, the number of cases of DILI reported in Japan increased more than 10-fold in the period 1964-1973 compared to the previous decade. The current estimated incidence in the Western World is 1/50.000 treated patients and a recent French study found an annual incidence of 14 cases per 100.000 inhabitants. Therefore, DILI has become an important issue in the spectrum of liver diseases and a considerable proportion of needle liver biopsies are currently taken in the setting of (suspected) DILI.

The risk of DILI is higher in females and older people; pre-existing liver disease is generally not a risk factor, with chronic hepatitis B and C as an exception. Genomic studies are currently exploring individual genetic susceptibility to liver injury caused by specific types of drugs.

DILI is mediated by two chief mechanisms: intrinsic and idiosyncratic toxicity. Intrinsic hepatotoxins cause hepatocellular damage in a predictable dose-dependent manner directly by the drug or indirectly by one of its metabolites. However, with the exception of acetaminophen, intrinsic DILI is clinically not very relevant, since it is caused by products such as carbon tetrachloride and other environmental toxins. In most cases, these products cause acute, coagulative necrosis that is mostly zonal and not accompagnied by inflammation or fibrosis.

The vast majority of drugs (which includes dietary supplements and herbal medicines) cause liver injury of the idiosyncratic type, meaning that the drug causes injury in an unpredictable fashion and with a less clear dose-dependency. This type of injury can broadly be subdivided into hypersensitivity-related DILI and toxic metabolite-dependent DILI. In the former, metabolites formed by the CYP450-system trigger an immune respons and inflammation, while in the latter, the metabolite causes damage to a component of the hepatocyte. Importantly, both mechanisms can occur together and can affect both the hepatocytes and the bile duct epithelial cells. Registries of DILI have revealed that anti-infectives, anticonvulsants and NSAIDs, followed by herbal products in some regions, are the most common cause of DILI.

Since DILI is an exclusion diagnosis, the clinician will consider a biopsy after ruling out other liver diseases, trying to get a morphological confirmation of his suspicion. The role of the pathologists is to investigate this based on the evaluation of morphological patterns that are associated with the different types of DILI. Furthermore, other concomitant liver diseases can be excluded or investigated and sometimes the diagnosis will be made by the pathologist when the clinician did not consider or expect DILI.

Zonal damage accentuated around the centrolobular vein is one of the hallmarks of DILI caused by many drugs and is related to the zonal distribution of the CYP450-system and some membrane transporters of bile constituents. The damage is mostly clearly delineated from the surrounding parenchyma, causing a punched-out impression.
Another important feature is bilirubinostasis and when it occurs without inflammation or necrosis (so-called bland bilirubinostasis), it is highly suggestive of DILI. Interference of drug metabolites with transporters of bile products is the mechanisms causing this picture.
In cases were hypersensitivity is involved, a viral-like hepatitis with variable numbers of portal eosinophils will be seen most frequently. Toxic metabolite-dependent DILI can also cause a similar picture, with portal, interface and lobular inflammation. When these changes are accompagnied by bilirubinostasis, the term cholestatic hepatitis can be applied and the presence of bilirubin droplets in this setting reflects decreased liver function, causing its assocation with a worse prognosis.
Drug-induced auto-immune hepatitis (DIAH) can give a picture that is virtually identical to idiopathic auto-immune hepatitis, although in some cases the morphology is discrepant with the clinical picture. The incidence of this type of DILI appears to be increasing; further studies are needed to better elucidate the morphological features of DIAH. Compared to idiopathic DILI, DIAH seems to respond better to steroids.
As mentioned previously, DILI can also affect bile ducts, causing bile duct inflammation and damage or even a PSC-like picture. Again, the mechanism can be toxic metabolite-dependent or via the immune system. A cytokeratin 7 staining is helpful, since it will highlight the bile duct damage and reveal is severity through the extent of so-called cholate stasis. Bile duct injury recuperates more slowly than parenchymal injury and can evolve towards vanishing bile duct disease, occasionally necessitating liver transplantation.
Several other morphological patterns that are less frequently seen can be associated with DILI and these comprise induction, anisokaryosis, lipofuscinosis, non-alcoholic steatohepatitis, phospholipidosis, macro/microvesicular steatosis, granulomatous hepatitis, vascular abnormalities and hepatic stellate cell hypertrophy.
This presentation aims to be a guide in the adequate recognition of all these different patterns of injury, which forms the key to the morphological diagnosis of DILI; since the different patterns are more or less correlated with certain types of medication, it is sometimes possible to pinpoint the specific drug causing the liver injury. A good clinicopathological correlation is therefore essential in this diagnostic process.

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Alcoholic liver disease: clinicopathological features and clues for differential diagnosis.

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Introduction

Alcohol is part of the human history and the production of fermented beverages dates back to more than 10000 years. It represents the oldest known form of liver injury and remains a major cause of liver disease worldwide.
Dose, duration and type of alcohol consumption are key factors in the development of alcoholic liver disease (ALD). However the individual response varies greatly because differences in genetic background give variable susceptibility to ethanol toxicity and also because co-existence of other liver diseases enhances liver damage. An intake of 40 g/day in men and 20 g/day in women is considered to be associated with a risk of developing cirrhosis, but in practice the great majority of people with alcoholic cirrhosis have a consumption typically exceeding 80 to 100 g/day.
Recent developments have identified some non classical alcohol-induced liver lesions and recognized alcohol as an important cofactor potentiating the effects of other chronic liver diseases such as non-alcoholic fatty liver disease (NAFLD), genetic hemochromatosis and chronic viral hepatitis. In this context, liver biopsy is still very helpful.

Clinico-pathological features

The morphologic spectrum of ALD varies from steatosis to cirrhosis and the classical lesions are grouped into the three following categories: uncomplicated fatty liver, alcoholic steatohepatitis and chronic hepatitis with fibrosis or cirrhosis. These categories are neither distinct clinico-pathological entities nor distinct stages of evolution of ALD and they may coexist in the same patient and in his/her biopsy. Steatosis develops very rapidly in the majority of people drinking heavily but is also rapidly reversible. Steatohepatitis appears in 20% to 40% of cases, after more prolonged alcohol consumption. Finally, 10 to 20% of people drinking excessively for many years will progress to cirrhosis.

Steatosis

Simple steatosis is the first and most common lesion to develop. Usually, fatty liver is asymptomatic and detected by chance in routine investigations. Hepatomegaly can be present. Liver function tests are normal or only slightly elevated. A high ratio of aspartate aminotransferase to alanine aminotransferase is in favor of ALD. Elevation in serum gamma glutamyl transpeptidase is also used to identify high alcohol consumption.
Steatosis occurs predominantly in perivenular area where the hepatocytes have the highest concentration of enzymes involved in alcohol metabolism. Progressively, steatosis will affect the whole lobule. It can be graded, as in NAFLD, as mild, moderate

or severe. It is completely reversible within 2 to 6 weeks in case of abstinence. The main pattern is macrovesicular steatosis although microvesicular and mixed patterns have been described. Patients with a mixed pattern of steatosis have been found to have a higher risk of progressing to advanced liver disease than those with pure macrovesicular steatosis. Lipogranulomas (chronic inflammatory cells surrounding a steatotic hepatocyte) can be seen. Simple steatosis is generally considered as a "benign" condition but the presence of fat within hepatocytes represents the substrate for the more severe forms of liver injury resulting ultimately in fibrosis. In support of this suggestion, it has been shown that the severity of steatosis in an index biopsy is predictive for progression to cirrhosis.

Steatohepatitis

A subset of patients will develop alcoholic steatohepatitis (ASH), the acute presentation of ALD. Classically, symptomatic patients have a background of known alcohol-related advanced liver disease and after a period of very heavy drinking present with fever, jaundice, vomiting, anorexia and fatigue together with signs of acute liver failure. Concomitant cirrhosis already exists in more than half of the cases. In severe forms, the short-term mortality rate may reach 50% and patients who survive progress to chronic fibrosing liver disease, almost half of them developing cirrhosis after 10 years. Serum transaminase levels are elevated and high peripheral white blood cell count with neutrophils predominance is a typical finding.

The specific histological features of alcoholic steatohepatitis are steatosis, hepatocyte ballooning and lobular inflammation, developing again predominantly in the centrilobular area. Steatosis is variable and can be mild or even absent in case of abstinence due to severe symptomatic liver disease. Hepatocyte ballooning is recognized by the presence of rounded and larger hepatocytes exhibiting cytoplasmic clarification, and formation of Mallory hyaline. Bright red giant mitochondria may also be found in ballooned hepatocytes. Lobular inflammation is made of a mixed population of inflammatory cells, typically rich in neutrophils clustered around ballooned hepatocytes containing Mallory hyaline (a phenomenon called satellitosis). Cholestasis is frequently observed. In contrast with other acute hepatitis, apoptotic bodies are rarely seen in ASH. Central sclerosing hyaline necrosis has been reported in some severe cases, with confluent hepatocyte necrosis, fibrous obliteration of sinusoids by hyaline sclerosis and numerous neutrophils, all in perivenular areas. ASH is associated with the development of centrolobular and perisinusoidal fibrosis which may progress to cirrhosis.

Fibrosis and cirrhosis

In ALD, activated hepatic stellate cells accumulate predominantly in centrilobular areas leading to perivenular fibrosis (sometimes referred as phlebosclerosis), perisinusoidal fibrosis (also seen when there is impaired sinusoidal blood flow that can also occur in case of excessive alcohol consumption) and to the highly characteristic pericellular fibrosis in which collagen surrounds individual hepatocytes giving rise to the so-called "chicken-wire" appearance also

found in NAFLD. If liver injury continues, these early fibrotic lesions extend, forming central to central and central to portal bridging. Initially cirrhosis related to ALD exhibits a micronodular pattern that can evolve into a mixed pattern of nodularity or sometimes a macronodular type of cirrhosis. If alcohol consumption persists, macrovesicular steatosis or ASH can be seen on a background of cirrhosis whereas in case of abstinence, the etiology of cirrhosis may no longer be obvious histologically.
Established cirrhosis occurs in case of long-lasting excess of alcohol consumption over a period of many years. Cirrhosis resulting from ALD has a mortality rate of approximately 50% at 5 years but the prognosis is better in case of abstinence. Clinical features are the same as those observed in other causes of cirrhosis and ALD patients develop the same complications. Amongst them, there is a risk of developing hepatocellular carcinoma (HCC) in ALD not only because of cirrhosis but also because alcohol seems to promote hepatic carcinogenesis. Therefore alcohol-associated HCC may also develop in precirrhotic liver. The risk of HCC is increased if co-existing liver diseases are present.

Other histological changes

Ductular reaction as well as portal inflammation with mild interface hepatitis can be seen in ALD and give rise to periportal fibrosis. If particularly prominent however they should raise the possibility of the presence of a co-existing disease such as viral hepatitis or impaired bile flow, for example in case of alcoholic chronic pancreatitis.
Mild iron overload is a common finding in ALD, occurring predominantly in hepatocytes. Importantly, iron acts as a co-factor increasing liver damage. In cirrhosis the deposits can be more abundant with a preferential periportal distribution that mimics genetic hemochromatosis.
Changes in hepatocytes include ground-glass hepatocytes, oncocytic appearance of their cytoplasm and presence of PAS positive diastase-resistant globules corresponding to glycoproteins or to α1-antitrypsin accumulation.

Differential diagnosis and interactions with other liver diseases

Fat predominating in zone 3 is found in ALD, in obesity and diabetes mellitus (NAFLD resulting from the metabolic syndrome), in different causes of malnutrition and malabsorption and in case of treatment by corticoids. Steatosis occurs in hepatitis C but predominates in periportal areas except in genotype 3 infection in which steatosis is more important and may affect the whole lobule.
ASH share histological features with non alcoholic steatohepatitis (NASH) observed in the metabolic syndrome, in drug-induced liver disease (amiodarone, perhexilene maleate…) and in Wilson disease. Ballooning and Mallory hyaline are also observed in chronic cholestatic syndromes.
In case of drug-related injury, Mallory hyaline may be very numerous with only mild fatty changes and a periportal rather than perivenular distribution. Hepatocyte ballooning and Mallory hyaline are also found predominantly in periportal area in Wilson disease in which glycogenated nuclei and inflammation are also present but pericellular fibrosis is rarely seen.

Commonly, distinction between ALD and NAFLD is not possible on morphologic grounds alone and relies on clinico-pathological correlation with the cut-off value of alcohol consumption set at 20 to 40 g/day. Mallory hyaline and neutrophils are more prominent in ASH than in NASH whereas more severe fatty changes and nuclear vacuolization indicative of insulin resistance are more prominent in NASH. Fibrosis progresses in a similar manner in both diseases although zone 3 pericellular fibrosis is more pronounced in ALD and perivenular fibrosis and phlebosclerosis are rarely seen in NAFLD.

There are important interactions to know between ALD and a number of other liver diseases. These include NAFLD, genetic hemochromatosis, viral hepatitis and drug-induced liver injury. Many people who drink excessively also have risk factors for the metabolic syndrome that can be potentiated by alcohol and the reverse is also true. Assessing their relative roles in histological changes may be difficult. Iron accumulation potentiates the liver lesions in ALD patients and patients who suffer from genetic hemochromatosis have an increased risk of progressing to cirrhosis in case of high alcohol consumption. People drinking heavily are also more at risk to be affected by viral infections and those who will have chronic viral hepatitis B or C are more likely to develop progressive liver damage. There can be some overlap between the histologic changes occurring in ALD and in chronic viral hepatitis, particularly hepatitis C virus infection. If portal inflammation is the main finding, chronic viral hepatitis is the predominant factor. Finally, chronic alcohol consumption induces cytochrome P450 2E1 (CYP2E1), which plays an important role in the metabolism of alcohol. Patients are therefore more susceptible to toxic injury from drugs metabolized by the same pathway. The most important example is paracetamol (acetaminophen), which can cause serious liver injury in chronic alcoholics at much lower doses than those required to induce hepatotoxicity in other individuals.

Treatment for ALD is abstinence. Steatosis disappears first (within a few weeks), followed by hepatocyte ballooning and inflammation (usually within 3 months). Occasional Mallory hyaline may persist for several months. End-stage ALD is a common indication for liver transplantation. Most transplant centers require a period of abstinence of at least 6 months before being listed for liver transplantation. Not surprisingly, therefore, features of steatosis or steatohepatitis are rarely prominent in these hepatectomy specimens. Small foci of pericellular fibrosis may persist and orientate to previous ALD. Up to one third of patients go back to drinking after transplantation. Liver transplant patients are also at risk for developing a metabolic syndrome with the histological features of NAFLD and this differential diagnosis has to be taken into account in post-transplant liver biopsies.

AASLD practice guidelines on ALD *(O'Shea RS et al. Hepatology, 2010)*

Recommendations on the role of liver biopsy

"For patients with a clinical diagnosis of severe AH for whom medical treatment is contemplated, or for those in whom reasonable uncertainty exists regarding the underlying diagnosis, a liver biopsy should be considered. This decision will depend on local expertise and ability in performing a liver biopsy in patients with coagulopathy, the patient's severity of illness, and the

type of therapy under consideration. (Class I, level C).”

A liver biopsy can indeed be useful in establishing the diagnosis of ALD because up to 20% of patients have another coexisting cause of liver injury. In addition, in the absence of decompensated disease, biopsy helps in the evaluation of the severity of ALD.

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Drug-related pathology of the gastrointestinal tract

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A large number of drugs can cause gastrointestinal side-effects with clinical symptoms ranging from diarrhea, constipation, nausea or vomiting to ulceration, bleeding or perforation (1). One drug can be responsible for different clinical presentations and variable pathological findings. As these microscopic injury patterns are often not specific or pathognomonic, making a correct diagnosis of drug-induced pathology can be very difficult. Moreover, pathologists are usually not informed about the drugs that a patient has been taking, although this information together with the clinical history are essential in establishing the diagnosis. The possibility of a drug-related aetiology should be considered, especially in cases of unusual pathology without apparent explanation (1). Drug-induced damage is mostly a reversible condition if drug intake is stopped, emphasizing the importance of recognizing this pathology (1,2).

Demonstrating that the drug is the cause of the pathological findings, is difficult. A correlation can be suggested if there is a temporal relationship between the start of the drug and the onset of the clinical symptoms and pathological changes and if these features regress when the drug is withdrawn. A subsequent challenge with the drug and re-appearance of the symptoms can prove a causal relationship, but is often not applied in daily clinical practice (1,2).

The major injury patterns of drug-induced gastrointestinal disease include ulceration, stricture formation, variable inflammatory processes and ischaemia. These non-specific microscopic patterns can display certain features and clues, indicating that a drug-induced aetiology should be taken into account: apoptotic epithelial cells, cytoplasmic vacuolation, increased intra-epithelial lymphocytes, melanosis coli, eosinophils (1,3). In addition to these 'de novo' disease patterns, drug-induced damage can present as an effect on pre-existing disease, again complicating establishing a correct diagnosis (2).

Non-Steroidal Anti-Inflammatory Drugs (NSAID) are one of the main classes of agents involved in drug-related gastro-intestinal disease. NSAID can provoke damage at all levels of the gastrointestinal tract. Its function as an inhibitor of prostaglandin synthesis is the main pathogenetic mechanism in causing mucosal ulcerations in the stomach en colon, but recent findings suggest another pathophysiology in small intestinal injury involving direct cytotoxic actions of NSAID on enterocytes in combination with bile and enterohepatic recirculation (4,5). NSAID are a common cause of pill-oesophagitis, a condition that is seen secondary to local caustic injury when retention of a pill in the (usually mid) oesophagus occurs. In the stomach, up to 45% of NSAID patients will develop a reactive gastropathy (6). NSAID erosions are mainly located in the gastric body and heal within days, whereas NSAID ulcers that are often large and multiple and located in the gastric antrum, tend to be chronic and more susceptible for

complications such as bleeding and perforation (6). In the lower gastrointestinal tract, NSAID-related erosions and ulcers are usually seen in the distal ileum, but new imaging modalities such as capsule endoscopy show that erosions in the small intestine are more common than previously appreciated (5). As in the stomach, these ulcerations are non-specific, only the absence of inflammation or other pathology in adjacent, non-ulcerated mucosa can draw the pathologist's attention to the possibility of a drug-induced origin. Eosinophils may predominate in the inflammatory infiltrate, but this is a general feature of drug pathology and not specific for NSAID (1). 'Diaphragm disease' is believed to be pathognomonic for NSAID use. It was first reported by Lang et al. in the small intestine. They described narrowing of the lumen by multiple, concentric septae-like projections (7). This pathology can also be located in the right colon, especially in patients taking sustained release preparations of NSAID (8). Biopsy material is non-specific and diagnosis is based on macroscopy. Other presentations of NSAID damage in the colon are (focal) active colitis, microscopic colitis (lymphocytic and collagenous type) and increased epithelial apoptosis (2).

Proton Pump Inhibitors (PPI) block gastric acid production and induce hypergastrinemia which has a trophic effect on enterochromaffin-like cells, resulting in linear or micronodular endocrine cell hyperplasia and on parietal cells, resulting in parietal cell hyperplasia, characterized by parietal cell extension up to the level of the foveolar neck region and enlarged, crowded parietal cells with luminal bulging within the deeper glands (9,10). Fundic gland cysts may arise, presumably due to obstruction of acid flow by hypertrophic parietal cells (10). The association between PPI use and fundic gland polyps is controversial and extensive prospective studies on this relationship are lacking. Recent studies found that PPI use is associated with an increased risk of community acquired *Clostridium difficile* colitis, although **antibiotics** are the best known risk factor for this colonic injury pattern (11). Also less severe forms of active colitis can be seen in association with the use of antibiotics.

Sometimes more specific microscopical findings point towards an iatrogenic origin of gastrointestinal disease. This is the case when **Kayexalate** crystals are recognized adherent to mucosal ulcerations or perforations. Kayexalate is a cation exchange resin, given in the treatment of hyperkalemia and typically administered with sorbitol, an osmotic laxative. The latter component is believed to cause the ischemic colonic necrosis associated with this drug (12). Upper gastrointestinal injury is less common and usually less severe (13). Brown crystalline material can be found in lamina propria or surface exudates of patients consuming **iron** tablets. Iron causes erosions and ulcerations of the oesophageal and gastric mucosa (14). Another specific finding is the presence of lipofuscin-type pigment in macrophages of the lamina propria of the colon, termed 'melanosis coli' or 'pseudolipofuscinosis coli'. This drug-induced phenomenom can occur after long-term use or abuse of **anthraquinone-containing laxatives**. The active compound of these contact laxatives binds to surface epithelial cells and promotes apoptosis of these cells; drug metabolites and cell remnants are then engulfed by the macrophages, resulting in the typical colour. The epithelial cell damage

is usually associated wih mild mucosal inflammation (2). When pseudodysplastic changes with nuclear atypia, pseudostratification and loss of polarity are observed in gastrointestinal biopsies, this should be recognized as typical characteristics for certain **chemotherapeutical agents, colchicine or cyclosporin** (2,14).

TABLE : Drug-induced injury patterns in upper and lower gastrointestinal tract

Oesophagus and stomach

Non-specific oesophagitis/gastritis
KCl, NSAID, alendronate, doxycycline, quinidine, iron, kayexalate, chemotherapy, corticosteroids

Reactive gastropathy
NSAID

Parietal cell changes and fundic gland cysts/polyps
PPI

Deposition of crystalline material
kayexalate, iron

Gastric mucosal calcinosis
antacids, H2-receptor antagonists, PPIs in renal transplant/failure patients

Pseudodysplastic changes
Hepatic Arterial Infusion Chemotherapy (HAIC), Selective Internal Radiation (SIR), colchicine, taxol

Epithelial apoptosis
PPI, colchicine

Small and large intestine

Erosion and ulceration
NSAID, KCl, iron, kayexalate, colchicine, ergot alkaloids, local analgesics (e.g. in suppositories)

Strictures and diaphragms
NSAID, KCl, pancreatic enzyme replacement

Perforation
corticosteroids, contrast media

Acute colitis
NSAID, antibiotics, carbamazepine, oral contraceptive steroids, laxatives, NaPO4, glutaraldehyde

Focal active colitis
NSAID, NaPO4

Pseudomembranous colitis
antibiotics, PPI, chemotherapy

Neutropenic enterocolitis
cytosine arabinoside, cisplatin, vincristine, adriamycin, 5-FU, mercaptopurine

Opportunistic infections
immunosuppressive agents, corticosteroids

Malakoplakia
: corticosteroids

Ischaemic-type colitis
: cardiovascular drugs (digoxin, alpha-adrenergic blockers, antihypertensive drugs, diuretics), oral contraceptive steroids, cocaine, ergot alkaloids, vaspressin and other vasoconstrictors, neuroleptics, NSAID, sumatriptan, alosetron hydrochloride, glutaraldehyde, flutamide, α-interferon

Microscopic colitis
: PPI, NSAID, H2 receptor antagonists (ranitidine, cimetidine), ticlopidine, simvastatin, flutamide, carbamazepine, paroxetine, sertraline, penicillin V, veinotonics (Cyclo 3 Fort), vinbumine, ferrous sulphate, levodopabenserazide

Inflammatory bowel disease-like colitis
: gold salts, NSAID, aminogluthemide, mycophenolate mofetil

Graft-versus-host-like disease
: mycophenolate mofetil

Epithelial apoptosis
: NSAID, NaPO4, anthraquinones, 5-FU, irinotecan

Pseudodysplastic changes
: cyclosporin

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SAFETY PROFILE AND HEPATOTOXICOLOGICAL DIFFERENCES OF YONDELIS® BETWEEN SPRAGUE DAWLEY RATS AND CYNOMOLGUS MONKEYS

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Introduction: Preclinical development and the role of the Toxicologic Pathologist
Well designed and executed preclinical studies are critical to the success of any drug development program. They must reliably assess the safety of a new drug entity, laying the groundwork for clinical trials and ultimately, regulatory approval. Safety testing of new pharmaceuticals is mainly based on international guidance documents and health authority regulations (e.g. : Guidance M3 – *Nonclincal Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals,* FDA http://www.fda.gov/cder/guidance, or EMA http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/landing/regulation.jsp&mid). Topics include various aspects of safety (e.g. reproductive, carcinogenicity and genotoxicity), ADME (e.g. bioanalytical, pharmacokinetics and toxicokinetics), and safety pharmacology.
Toxicity studies need to be performed in rodent and non-rodent species and the longer the duration of the preclinical studies, the longer the period is granted for clinical trials.

Clinical Development Trials
Repeated-dose toxicity studies in two species (one non-rodent) for a minimum duration of 2 weeks (see Table 1) would generally support any clinical development trial up to 2 weeks in duration. Clinical trials of longer duration should be supported by repeated-dose toxicity studies of at least equivalent duration. Six month rodent and 9 month non-rodent studies generally support dosing for longer than 6 months in clinical trials.

Table 1. Recommended Duration of Repeated-Dose Toxicity Studies to Support the Conduct of Clinical Trials

Maximum Duration of Clinical Trial	Recommended Minimum Duration of Repeated-Dose Toxicity Studies to Support Clinical Trials	
	Rodents	**Non-rodents**
Up to 2 weeks	2 weeks[a]	2 weeks[a]
Between 2 weeks and 6 months	Same as clinical trial[a]	Same as clinical trial[a]
> 6 months	6 months[a]	9 months [a]

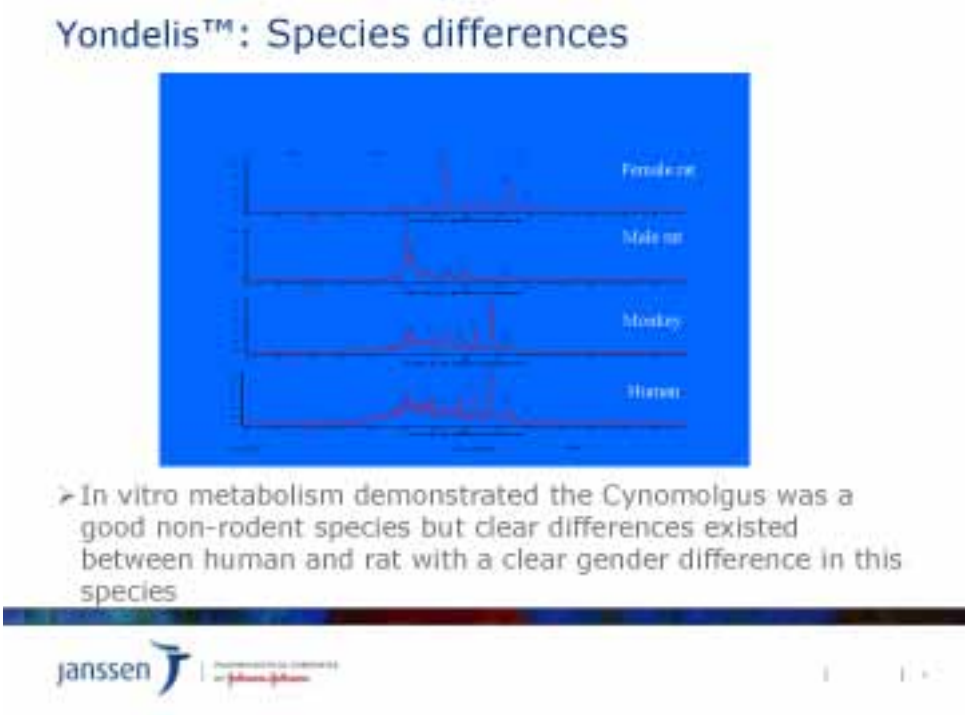
a. Exeptions/ details are listed in the guidelines

In these preclinical studies, the toxicologic pathologist needs to very accurately describe the lesions, tries to find the underlying pathogenesis behind the observed lesions and needs to be capable of discrimination of toxic/drug-induced changes from secondary or spontaneous lesions, the integration of biomarkers, and judging the possible relevance to humans and the associated impact on compound development.

Case study: Yondelis Safety Profile and Hepatotoxicological differences of Yondelis® between Sprague Dawley rats and cynomolgus monkeys

Yondelis® (trabectedin, ET-743) is a tetrahydroisoquinoline compound isolated originally from the marine ascidian *Ecteinascidia turbinata*. Yondelis® is indicated for the treatment of patients with relapsed ovarian cancer and for the treatment of patients with advanced soft tissue sarcoma, after failure of anthracyclines and ifosfamide, or who are unsuited to receive these agents, and in combination with Doxil® (pegylated liposomal doxorubicine hydrochloride) for the treatment of patients with relapsed ovarian cancer. Efficacy data are mainly based on liposarcoma and leiomyosarcoma patients. In patients, the most prevalent drug-induced toxicities are fatigue, non cumulative neutropenia and reversible transaminase increase.

In rats and monkeys, the principal toxicities were myelosuppression, local intolerance at the injection site, hepatotoxicity, intestinal epithelial atrophy and ulcerations.
In order to better understand the hepatotoxic potential of Yondelis®, its safety was evaluated in repeated dose studies in rodent (Sprague-Dawley rats) and non-rodent models (Cynomolgus monkeys).
Cynomolgus monkeys were chosen as the preferred non-rodent species due to the similarities in metabolic profile to that of humans. The dosing schedule in these preclinical studies should mimic the intended dosing schedule in patients. Yondelis® was administered via a 3-hour and 24-hour intravenous infusion every 3 weeks for 3 (rat), 4 and up to 8 (monkey) cycles.



After repeated administration in rats, mortality was observed at 50 µg/kg in females and at 50-75 µg/kg in males. A pronounced dose-dependent and only partially reversible hepatotoxicity (transaminase increase, inflammation, hepatocellular necrosis and bile duct proliferation, cholangitis) was noted, and toxicity was cumulative and more pronounced in female rats. The difference in gender sensitivity in rats is likely to be linked to differences in the metabolic profile, biliary excretion and/or liver retention.

In Cynomolgus monkeys dosed up to 120 µg/kg, a similar toxicity profile was seen in both sexes, comprising myelosuppression and dose-dependent local intolerance at the injection site. Hepatotoxicity was less pronounced than in rats and non-cumulative in nature (transaminases increase, hepatocellular hypertrophy, hepatocellular degeneration/necrosis and mixed inflammatory cells in the sinusoids and portal tracts). At 50 µg/kg, exposure to Yondelis® was however higher in monkeys (23-28 µg.h/l) than in rats (1.7-8.6 µg.h/l), without gender difference.

These data suggest that the Cynomolgus monkey is a more relevant and predictive model for human Yondelis® hepatotoxicity than the rat.

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Anatomic Pathology in the Digital Era

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It is unlikely that anatomic pathology can escape entering the digital era: this lecture is based on this assumption. Several applications are already more and more widely used for clinical and for pharmaceutical research purposes (to name a few: initial and continuous education in pathology, archiving of reference cases, quantification via image analysis, second opinion / peer review). However the possibilities of digital pathology are far broader than what was done up to now and can rejuvenate our 150 year-old discipline: some examples of these revolutionary means will be presented to support this view.

In a first part, the installation of a digital pathology system within Janssen R&D will be presented as an example of a state-of-the-art implementation. Five research sites, located all over the world(Belgium, China, France, USA), are equipped with a slide scanner and with high quality monitors. The slides are available to these 5 sites but also to authorized users outside the Janssen R&D network, via a secure cloud server available 24/7 worldwide in any web browser. Special attention will be given to the issues encountered during the implementation (specifications, budget and recurring costs, standardization of histotechnique, quality of scanner and of display, ergonomics, performance of the network and effect on efficiency).

Two image analysis examples, still work in progress, will then be presented to illustrate advanced uses that can bring quantitative results with an efficiency that cannot be matched by standard toxicological pathology. The first application is the automatic recognition of the rat kidney architecture (including cortex, glomeruli, outer and inner stripes of the outer medulla, inner medulla, papilla and stroma), so that a feature of interest (for example a Ki-67 IHC staining) can be quantified by region and not on the whole kidney, in an unsupervised way. The second application is the quantification (number, shape, volume) of corpora lutea in mice ovaries, which requires to prepare about 45 sections per ovary, or 90 per animal. Given the highly time-demanding manual evaluation, using an automated stereological method is highly desirable, in order to correctly assess the endpoint chosen.

The three examples presented above can be gathered under the broad concept of Computer Aided Histopathology (CAH), a new field for pathology. Interested pathologists could join this “sub-specialty”, so that pathology can keep pace with the digital era, and that other colleagues can benefit from the new tools and knowledge. Among those tools, the European pathologists could create an association similar to the US Digital Pathology Association (DPA) so that there is a forum for sharing the knowledge and leading this new field.

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Update in Intraductal Papillary Mucinous Neoplams

Nathalie Nagy (CHU Charleroi)

Introduction

Intraductal papillary mucinous neoplasms (IPMN) are grossly and radiographically visible epithelial tumours arising from the main pancreatic duct or the duct branches. They cause duct dilatation and mucin production. Their incidence is poorly defined because most are asymptomatic but they have been reported to account for approximately 7% of clinically diagnosed pancreatic neoplasm and up to 50% of incidentally detected pancreatic cysts. IPMN represent approximately 25% of pancreatic cystic neoplasms and account for up to 5-20% of pancreatic resections for malignancy.[1, 2]

First described in 1982 by Ohashi, their incidence has increased in the last two decades but in the absence of an increase in IPMN-related or overall pancreatic cancer-related mortality, this increase seems more due to diagnostic scrutiny, than greater numbers of patients with clinically relevant disease. [3]

Aetiology

There are no well-established environmental etiological factors. The incidence of synchronous and metachronus neoplasms of other organs appear to be high, mostly colorectal and pancreatic ductal adenocarcinoma. Recently an association between IPMN and familial pancreatic cancer has been suggested. The most common identified germline mutation leading to an increased risk of pancreatic cancer is in BRCA2 gene found in 15-20% of familial pancreatic cancer families, recently described in a small number of patients with IPMN. Another germline mutation associated with IPMN is of the family of mismatch repair (MMR) gene. Moreover, IPMNs have been reported in patients with Peutz-Jeghers syndrome and in patients with familial adenomatous polyposis.[4,5]

Clinical features

IPMN mainly occur in the sixth to seven decades of life rarely before the age of 40, affecting slightly more frequently males. The majority of IPMN occur in the head of pancreas, but location is not specific. They may manifest as recurrent pancreatitis with and without hyperamylasemia, steatorrhea, diabetes and weight loss or may be entirely asymptomatic and are incidentally detetected.[1, 2]

Imaging

IPMN can be classified as main duct IPMN or branch duct IPMN by imaging and since main duct IPMN and branch duct IPMN have significant differences in prevalence of cancer ranging from 57 to 92% and 6 to 46%, respectively, the correct classification by imaging is of major implication. On computed tomography (CT) or magnetic resonance cholangiopancreatography (MRCP) dilatation of the main duct ≥ 1cm strongly suggest main duct IPMN, whereas a presence of a pancreatic mucinous cyst communicating with the pancreatic duct without duct dilatation suggests branch duct IPMN. The presence of papillary

growth in branch or main ducts can be ascertained with endoscopic retrograde cholangiopancreatography (ERCP). MRCP has been reported superior to CT in the diagnosis of IPMN. [6]

EUS FNAB and fluid analysis

The sensitivity and specificity of cytological diagnosis and grading of IPMN are both low. Some cytological features as prominent nucleoli and para chromatin clearing seems to be more frequent on high-grade IPMN. Thigh epithelial cell clusters were also observed in the IPMN with at least intermediate dysplasia. The presence of necrosis and inflammation in the background was found to be associated with the presence of an invasive carcinoma. EUS-FNAB is at least helpful in differentiating high-grade from low-grade IPMNs.

Cyst fluid carcinoembryonic antigen (CEA) levels are generally though to be very helpful in distinguishing mucinous cystic lesion from non-mucinous cystic lesion of the pancreas. However, CEA and Ca19-9 levels are not as useful in assessing the degree of dysplasia in an IPMN. Nevertheless, determination of serum levels of CEA and CA19-9 may aid in the differentiation of invasive from non-invasive IPMN.

Pancreatic cyst fluid DNA analysis (KRAS2 gene mutation, allelic loss of tumour suppressor gene such as TP53, p16/CDNK2A and PTEN) have also been performed but remain to be developed to guide the management of suspected IPMNs.[7]

Macroscopy

IPMNs are intraductal grossly visible (≥ 1cm) epithelial neoplasm of mucin-producing cells.

Main-duct type IPMN

In this type the main pancreatic duct is diffusely dilated. The duct is often filled with mucin and is tortuous and irregular. In some cases mucin extrude from the ampullary orifice. Most of them involve the head of the gland, approximately one third are localized to the body and tail, and occasionally the entire main duct is involved. Approximately 60% have high-grade dysplasia and 45% are associated with an invasive carcinoma. [7,8]

Branch duct type IPMN

This type arises most often in the uncinate process. They form multicystic, grape-like structures filled with mucin. The cyst walls are usually thin, and can have either a flat or papillary lining. The cysts will communicate with the main pancreatic duct but the neoplastic growth does not significantly involve the main duct. Multiple distinct lesions are seen in more than one third of cases. Most are low grade with very indolent behaviour.[7, 8]

Mixed IPMN

Mixed-subtype IPMNs involve both main duct and branch ducts and have similar clinical presentation and biological behaviour to the main duct type. [7]

Sendai criteria

International consensus guidelines for management of IPMNs were reported in 2006 and give some indication for resection. Since the frequency of malignancy in main duct type IPMN has ranged from to 60 to 92%, and that approximately two-thirds

of these have been invasive, it is recommended to resect all main duct and mixed variants of IPMNs as long as the patient is a good surgical candidate with a reasonable life expectancy. Regarding the branch duct type, with only 6 to 46% prevalence of carcinoma, some criteria were put forth to guide which branch duct type were safe to observe and which should be resected. Surgical resection of branch duct type IPMNs is indicated in patients with symptoms, cyst size ≥ 3cm, intramural nodules, dilated main pancreatic duct greater than 6mm and positive cytology[6, 7,9,10]. The follow-up of isolated branch duct cysts by imaging (CT and MRCP) could be made every two years if lesion is less than 1cm, every one year if bigger.[11]

Microscopic features

IPMNs are characterized by the intraductal proliferation of columnar mucin-producing cells. IPMNs lack the "ovarian-type" hypercellular periductal stroma typically seen in mucinous cystic neoplasms. Architecturally, the epithelium of IPMNs can be flat or form papillae with fibrovascular cores. These lesions are distinct from pancreatic intraepithelial neoplasia (PanIn), which is a microscopic lesion that is usually not macroscopically detectable. The lesions can be focal, multifocal (>40% of cases) or diffuse. Non-invasive IPMNs show a spectrum of lesions from low-grade dysplasia (adenoma), to intermediate grade (borderline) or high-grade dysplasia. 30% of IPMNs have an associated invasive carcinoma. Four main pathologic types are described.

Gastric foveolar-type IPMN

This type is usually flat with a single layer of the cells lining dilated ducts. Gastric foveolar type is almost always a low-grade lesion and involves the vast majority of branch duct IPMNs.The neoplastic cells often extend along the duct system into the adjacent pancreatic parenchyma, producing acinar-ductal metaplasia, acinar atrophy and fibrosis. The lining epithelium typically does not express MUC1 and MUC2 but well MUC5AC and MUC6 supporting their gastric differentiation. Genetic analysis reveal a significantly higher frequency of KRAS mutation as well as ERK status close to that seen in conventional pancreatic ductal adenocarcinoma in gastric foveolar type than in intestinal type.[12]

Intestinal-type IPMN

Intestinal-type resembles villous adenomas of the colon. This type has either intermediate or high-grade dysplasia. The epithelium does not express MUC1, weakly express MUC5AC and MUC2. Epithelium also expresses CDX2 suggesting an intestinal differentiation. When associated with an invasive carcinoma most are colloid carcinomas.

Pancreatobiliary-type IPMN

This type is generally composed of cuboidal neoplastic cells, more atypical containing less mucin than do the cells in intestinal-type. The pancreatobiliar-type forms more complex papillae with bridging and cribriforming. They therefore tend to be high-grade lesions. The immuno-histolabeling pattern is similar to that of pancreatic intraepithelial neoplasia. They express MUC1 and MUC5AC but not MUC2. Some also express

MUC6. Invasive associated carcinomas tend to be classic tubular-type ductal carcinoma.

Intraductal oncocytic papillary neoplasm (IOPN)

Architecturally, IOPN are distinct from others IPMNs, they are very complex with arborizing papillae, cribriform formations and solid nests. Mucin-containing intraepithelial and intra-acinar lumina are frequently seen. Because of their markedly atypical cytoarchitecture most are classified as having high-grade dysplasia. IOPNs express MUC1 and MUC6, MUC2 and MUC5AC is usually restricted to goblets cells scattered distributed into the lining epithelium.

Intraductal tubulopapillary neoplasm (ITPN)

Rare neoplasm accounting for <1% of all exocrine neoplasms and only 3% of intraductal neoplasms of the pancreas, this is the most recently recognised and the most controversial of all of the IPMNs. ITPNs can be distinguished by their more solid growth without visible mucin, scanty cytoplasmic mucin, tubulopapillary architecture, frequent necrotic foci and absence of KRAS2 gene mutation. Uniform high-grade dysplasia is observed throughout the lesion. Epithelium expresses MUC6 and focally MUC1 and is negative for MUC2 and MUC5AC. Grossly, ITPN forms solid, nodular masses within pancreatic ducts. The nodules are large and mucin secretion is not present. The surrounding parenchyma is often sclerotic. Associated invasive carcinoma is found in about 40% of cases and the invasive component is usually limited in extend. According to the WHO classification, IPM carcinoma (IPMC) is classified as either "non invasive" or "invasive". Non-invasive IPMNs show a favourable postoperative outcome in comparison with invasive IPMC, with a 5-yaer survival rates ranging from 77 to 100%. Minimally invasive IPMC has been categorized within the classification of pancreatic carcinomas used by the Japan Pancreatic Society since 1993. Patients with IC-IPMC had a significantly worse outcome than those with MI-IPMC and there was no difference in postoperative outcome between patients with MI-IPMC and those with non-invasive IPMC. [13] In the series of Nara et al, none of the patients with MI-IPMC showed lymph node metastasis and implies that complete resection of the lesion without lymph node dissection may be sufficient for the treatment of MI-IPMC. It is thus of great interest to classified this carcinomas preoperatively. Multidetector row computed tomography was found to be useful to distinguish IC-IPMC from MI-IPMC and non-invasive IPMNs with more than 80% sensitivity and 100% specificity. MI-IPMC are characterized by:

-an infiltrative distance of 5mm or less is regarded as minimal invasion. Venous, lymphatic or neural invasion within the area of ≤ 5mm of the infiltrative distance is also counted in this category. This includes the invasion of various histologic types.

-the mucous lakes not associated with mucinous carcinoma showing infiltrative growth are features of minimal invasion regardless the size and location of mucous lakes. If the mucous lakes contain scanty floating cancer cells, it is described as mucous rupture with cellular component including a kind of pure mucinous carcinoma associated with IPMC. If many cancer cells are floating

in mucus lakes it is treated as infiltrative growth and the infiltrative distance of 5mm or less is regarded as minimal invasion.
-loss of the basement membrane of the pancreatic duct with IPMC is regarded as minimal invasion. If I-IPMC grows expansively, even if it ruptures into the bowel, or even if it erodes a major vessel wall unless cancer cells enter the lumen of the major vessel, it is still regarded as minimal invasion.
Finally, the subtype of associated carcinoma is also of interest. Two major types represent these associated carcinomas: invasive carcinoma mimicking usual invasive ductal adenocarcinoma and invasive colloid carcinoma. Patients with an IPMN associated invasive colloid carcinoma usually have a better prognosis than those with an associated invasive ductal carcinoma.[14]The gastric and intestinal types of IPMNs have less malignant potential than the pancreatobiliary type.[15]

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Cervical Cytology and HPV: a competition?

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The technique of cervical cytology has remained virtually unchanged since the publication of the idea in 1943[1]. Modifications have been made to automate and standardize specimen preparation and cytotechnologists may be aided by computer-guided screening, but the basics of the technique are unaltered. Cytology is still the cornerstone of cervical cancer screening programmes around the globe and these programmes have been very successful in preventing morbidity and mortality in women.
After the hypothesis had been formulated that the human papillomavirus (HPV) plays an important role in the cause of cervical cancer[2], the race was on to confirm this idea and elucidate the process of carcinogenesis. From this vibrant branch of research several important facts have emerged. A small group of HPV subtypes is indeed the causal factor in cervical cancer[3,4], even though its presence alone seems to be insufficient and co-factors are often needed during carcinogenesis.
The study into HPV has yielded a plethora of tests to identify the virus and its (oncogenic) subtypes. Vaccines have been developed against the most prevalent of the oncogenic subtypes, which have the highest cancer burden. This evolution has impelled clinicians and pathologists to think about the optimal way to implement and integrate new knowledge, tests and treatments in daily practice in order to improve patient management. In many disease processes it is routine to combine tests from pathology, molecular biology and genetics to yield an integrated diagnosis. In this way the prognosis of the patient becomes clearer and management can be tailored more specifically for each patient. Target testing of diseases caused by a specific agent or identification of cases that will respond best to a certain treatment illustrate this approach. Well-known examples of this can be found in the work-up of various malignancies and already it seems clear, that cervical cancer and its precursors will follow suit.
Despite the success of organized screening it is known that the sensitivity of cervical cytology is actually quite low. The test benefits from repetition at regular intervals. It is a very labour-intensive technique, requiring a high level of training and concentration. The expectation is, that these problems will exacerbate with the introduction of vaccinated women into the screening pool. HPV testing tackles the problem from a different angle, coming from molecular biology instead of morphology. Tests are probe-based and can be highly automated and computerized. Interpretation of results is less complex and much more standardized.
Several strategies have been proposed to combine HPV testing with cytology in order to gain sensitivity and better identify women at risk. Unfortunately the detection of oncogenic HPV DNA does not automatically imply the presence of a clinically relevant lesion (e.g. high-grade cervical dysplasia or worse), as most infections are transient and

not associated with symptoms. Combined with the high prevalence of the virus, this has a detrimental effect on the specificity of most HPV tests.
This presentation illustrates with several examples how cytology and HPV testing can be combined to make optimal use of the advantages of each test. This depends in part on the goal of testing, be it in screening, diagnosis or follow-up. The impact of a new paradigm on the workflow and personnel in the laboratory are not always well understood.
In screening a couple of approaches are being evaluated. A frequently used approach is to triage cytological abnormalities with an HPV test. Most screening programmes and recommendations include some kind of HPV triage, especially in equivocal cases[5]. In this set-up, the cytotechnologist does not know if the sample screened contains HPV. This information is added afterwards, perhaps after the clinician has received a provisional report from the lab. The cytology and HPV results can also be reported separately.
A second approach was taken in the Dutch POBASCAM trial[6], where the investigators performed both HPV and cytology. Either a positive HPV test or an abnormal cytology result was eligible for follow-up. Performed within the screening programme with a follow-up of 5 years, they showed that HPV testing significantly reduced detection of CIN 3 or worse in the second screening round relative to conventional cytology, with more high-grade lesions detected earlier. Additionally data also showed that HPV screening protected against cervical cancer better than cytology alone. On the basis of these findings the Health Council of the Netherlands issued advice to the Dutch minister of health to convert the screening programme to HPV primary screening. In such a programme, cytology would probably be used as a triage test.
When the HPV test is carried out before or in parallel with cytology, the cytotechnologist can be aware of HPV status whilst screening a slide. This can be used to enhance screening performance. In a study investigating this idea computer-guided cytological screening on liquid-based samples performed with prior knowledge of HPV status resulted in improved detection of CIN 2 or worse[7]. In the context of this same study it was also seen, that time management of the cytotechnologists was significantly different if HPV status was known, allowing them to spend more time screening difficult cases and recuperating this time while screening double-negative cases [own observations, not yet published].
A last approach is to co-test all samples [5]. Because of high cost and superfluous redundancy this is often not advisable for a screening programme. It may, however, be considered as an intake procedure for a screening programme, which provides a baseline for comparison in later stages.
The optimal combination is influenced by prevalence of both HPV and cervical disease in the population, the age of the target group, the level of HPV vaccination, costs of each test as well as costs in follow-up. Clinicians and laboratories should utilize cervical cytology screening paradigms that are most appropriate for their patient populations and clinical practice. These decisions should be continually re-evaluated as science

and technology evolve and as clinical studies provide scientific data on cost-effective strategies to further reduce morbidity and mortality from cervical cancer [8].
In the laboratory this requires a new approach and a new way of organizing. Cytotechnologists, laboratory technicians, pathologists and molecular biologists need to be aware of the advantages and restrictions of each test used in the process. Education for trainees, cytotechnologists and doctors needs to be adapted and updated to this end. By having the same people perform and evaluate both cytology and the HPV test, this kind of knowledge and expertise is created and sustained. This may require different laboratory departments to work more closely or even integrate part of their workflow. It will also lead to a more integrated diagnosis with more personalised advice on patient management.
As this process is ever evolving, there are still many unknowns. The clinical use to state HPV type and viral load as part of a medical protocol has not been studied thoroughly. The use of molecular markers in screening and diagnosis is not yet fully understood. Digital imaging is still improving but needs to overcome important obstacles as many systems lack speed and create burdensome large files in order to yield adequate images. It is this evolution that keeps the field vibrant and moving forward, with competition every once in a while, but mostly cooperation.

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ORAL PRESENTATIONS

ORAL PRESENTATIONS LIST

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ORAL PRESENTATIONS ABSTRACTS

O 01

A real-time PCR approach based on SPF10 primers for detection and typing of human papillomavirus

Micalessi MI (1), Boulet GA (1), Vorsters A (1), De Wit K (1), Jannes G (2), Mijs W (2), Ieven M (2), Van Damme P (1), Bogers J (1)

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The SPF10 PCR targets conserved primer regions of the HPV L1 gene enabling a broad spectrum amplification of a 65bp region for different HPV types. The resulting HPV amplicons can be subjected to the INNO-LiPA HPV Genotyping *Extra* assay (LiPA) to determine the exact genotype. This study aims to develop a SYBR Green-based real-time PCR to achieve simultaneous amplification and detection of the SPF10 target. This approach could reduce the workload and cost by eliminating the LiPA step for the HPV-negative samples.
The real-time PCR shows an analytical sensitivity of 29.7 copies for HPV6, 16, 18 and 31 and a HPV-specific melting peak with an average Tm of 82.2°C. Thirty-one HPV DNA plasmids representing different HPV types and four clinical samples were genotyped correctly using the SPF10 real-time PCR in combination with the LiPA assay. Here, the LiPA assay was performed at an increased hybridisation temperature (49.5°C) in combination with a reduced amplicon volume (1µl) to avoid cross-reactivity. In addition, the SPF10 real-time PCR-LiPA system allowed simultaneous type-specific detection of two genotypes up to a 100-fold excess of one genotype over the other, which indicated the feasibility of the detection of multiple infections.
In conclusion, the SPF10 real-time PCR proves to be very sensitive and generates amplicons which are compatible with the LiPA assay.

O 02

Colorectal Cancer in Young Cambodians

Monirath Hav (1,2), Sokha Eav (3), Vutha Ky (4), Claude Cuvelier (1), Sokneang In (5), Rithy Kong (6), Yana Kheang (3), Chakravuth Oung (2), Piet Pattyn (1), Dara Lem (3)

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[6] Siem Reap Provincial Hospital, Siem Reap, Cambodia

Introduction:
Colorectal cancer (CRC) is a common disease in the older population, but it has become increasingly evident that CRC is not infrequent in the young. The aim of this study is to describe the epidemiological, clinical and pathological characteristics of CRC in young Cambodians.

Materials and Methods:
We examined clinical and pathological data from all CRC cases registered in the two reference centres for gastrointestinal tumours in Cambodia between 2005-2010. Age-specific CRC incidence was computed using the national population census 2008 data from the National Institute of Statistics. We compared differences in distribution of tumour location, histology, differentiation and UICC/TNM stage in two age groups, namely < 40 and ≥ 40.

Results:
During this period, there were 356 new CRC cases, of which 29.78% affected patients younger than 40. This proportion is the second highest, with a higher proportion only reported in Egyptian population. The crude incidence was 2.82 and 2.36 per 100,000 in females and males, respectively. Adenocarcinoma was the most common histologic type, and $> 50\%$ of all tumours occurred in the colon, with no appreciable variation between the two age groups. Mucin-producing and advanced-grade tumours were twice more frequent in the young.

Conclusion:
The unusually high CRC proportion in the young in our study can be due to referral bias. Nevertheless, this matter, together with the continuous exposure to hazardous environmental agents and the prevalent consanguinity in Cambodia, justifies further research, which will advance our understanding of CRC risk factors and perhaps answer some questions concerning genetic-environmental interactions in CRC epidemiology in young adults.

O 03

Papillary thyroid cancer (PTC): Identification of a precursor lesion

M Lamba Saini, B Weynand, J Rahier, M Mourad, Marc Hamoir, Etienne Marbaix

UCL/ St Luc

Purpose/ Objective:
Papillary thyroid cancer (PTC) is the commonest endocrine malignancy. Though significant progress has been made to understand the pathways involved in the tumorigenesis of PTC, no precursor lesion has been identified till date. Present study aims to identify and understand the precursor lesion of PTC and its immunohistochemical (IHC) / molecular markers.

Materials/ methods:
Cases of PTC and its variants were retrieved from surgical pathology files (1990 till date). Cases of papillary microcarcinoma, follicular variant of papillary carcinoma (FVPTC) and metastatic PTC were reviewed. In addition, cases of a subset of follicular adenomatoid nodules that had focal areas with nuclear features characteristic of PTC, defined as follicular variant of papillary carcinoma in follicular adenomatoid nodule, were also analyzed. These lesions could be a possible precursor of FVPTC. IHC using a panel of thyroid malignancy markers including galectin- 3, HBME (Hector Battifora Mesothelial cell) -1, and cytokeratin (CK) 19
and proliferative markers like cyclin D1 and Ki 67 was performed to study the expression profile of the precursor lesion to evaluate whether it is intermediate between the benign and malignant groups. Slides were selected to include the tumor and a rim of normal thyroid. Tumors were then analyzed for cyclin D1 gene amplification by fluorescent in-situ hybridization (FISH).

PTC also frequently carries several genetic alterations in genes coding for proteins that are postulated to activate the extracellular-regulated kinase (ERK) pathway, a component of the Mitogen-activated protein kinases (MAPK) signaling pathway, which plays a key role in the regulation of cell growth, differentiation, and survival. We also evaluated the role of MAPK in PTC, particularly in FVPTC arising in a follicular adenomatoid nodule, by scrutinizing ERK phosphorylation in PTC and its variants by using IHC.

Results:
Ki 67 and cyclin D1immunolabeling showed increased proliferation in the precursor lesion of PTC and its variants. Amplification of cyclin D1 oncogene (CCND1, PRAD1) by FISH was significant in FVPTC arising in a follicular adenomatoid nodule and other PTC variants as compared to normal non malignant areas. The precursor lesions also showed immunolabeling of HBME-1, CK 19 and galectin-3. However, there was

absence of detectable ERK phosphorylation in the precursor lesion and also in the majority of tumor cells.

Conclusions:

Increased expression of cyclin D1 and amplification of its gene in areas of follicular adenomatoid nodules showing cytological features of PTC suggest that these areas could correspond to precursor lesion of FVPTC. Immunohistochemical labelling of Galectin 3, HBME-1 and CK 19, recognised markers of PTC, suggest that these lesions are indeed precursors of PTC. MAPK pathway signaling by activation of ERK is more complex than previously anticipated or there are alternate signal transduction cascades of MAPK signaling pathway involved in tumorigenesis of PTC.

PTC: Papillary thyroid cancer
IHC: Immunohistochemistry
FVPTC: Follicular variant of papillary carcinoma
MAPK : Mitogen-activated protein kinases
ERK : Extracellular-regulated kinase

O 04

Pathologic features, HER2 protein expression, and *HER2* and *CEP17* copy numbers in DCIS.

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Background
Previous studies in which HER2 status in ductal carcinoma in situ (DCIS) was evaluated yielded conflicting results. The reported prevalence of HER2 overexpression and amplification vary remarkably. These discrepancies might partly be due to differences in assessment methods, i.e. FISH or immunohistochemistry (IHC), and usage of tissue microarray or whole mount slides. To further investigate this issue, we performed FISH and IHC for HER2, evaluated *HER2* and *CEP17* copy numbers and correlated these data with histopathological characteristics.

Methods
In this study, 78 DCIS cases were included, of which 72 pure DCIS and 6 DCIS with micro-invasion. Analysis of the following histopathological features was performed: architecture, nuclear atypia, necrosis, calcifications, stromal inflammation, stromal morphology, extent of lesion, margin width, and pathologic classification according to Van Nuys Prognostic Index (VNPI). HER2 IHC and *HER2* dual probe FISH analysis were performed and scored according to ASCO/CAP guidelines. *HER2/CEP17* ratio and *HER2* and *CEP17* copy numbers were separately analysed, and presence of *HER2* clusters was noted. IHC for estrogen and progesterone receptor (ER and PR) was also performed. Whole mount slides were used for all analyses.

Results
Using HER2 IHC, 18 cases (23%) were scored negative (0 or 1+), 14 cases (18%) equivocal (2+) and 46 cases (59%) positive (3+). According to *HER2/CEP17* ratio, 44 of 77 cases (57%) showed *HER2* amplification; there was insufficient tissue for FISH in one case.
The amplification status of the DCIS lesions correlated with the IHC score ($p<0,001$). Of all 44 amplified cases, 39 (89%) were assigned a 3+ IHC score, and remarkably, all these cases but one showed HER2 clusters on FISH analysis.
Amplified lesions more often showed nuclear atypia grade 3 ($p<0,001$), extensive comedonecrosis ($p<0,001$), stromal inflammation ($p<0,001$) and myxoid stromal architecture ($p=0,037$). All lesions with micro-invasion were amplified ($p=0.034$). There was no correlation with patient age, hormone receptor status or other pathological variables.

In the amplified group, high-grade nuclear atypia was associated with a higher mean *HER2* copy number (p<0,001) and *HER2/CEP17* ratio (p=0,004), while this was not the case in the non-amplified group. *CEP17* copy numbers did not correlate with nuclear atypia.

Conclusions

The correlation between *HER2* amplification and adverse pathological features, including micro-invasion and high-grade nuclear atypia, underscore that HER2 is a driver of DCIS aggressiveness and possibly of recurrence as non-invasive cancer as well. However, the prevalence of HER2 overexpression, amplification and cluster formation was much higher than in invasive carcinoma, suggesting that HER2 might play a less important role in transition from DCIS to frankly invasive cancer. Further studies should evaluate non-invasive and invasive recurrence of resected DCIS separately.
Noteworthy, HER2 overexpression seems to evoke an immune response, since amplified lesions more often showed extensive inflammation in the surrounding stroma.

O 05

Histopathological parameters are associated with both severity and survival in alcoholic hepatitis.

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Background and aims: Alcoholic hepatitis (AH) which includes both histopathological features and a clinical syndrome is associated with increased morbidity/mortality. To date, no well-established histopathological criteria are linked to AH natural history. This study aims to evaluate whether histopathological parameters are associated with disease severity and survival in AH .

Methods: 170 patients (mean age 53 years, 62% males, mean MELD 15, median Maddrey [mDF] 32) with excessive alcohol intake (>40g/day) and biopsy proven AH were included. Histological parameters were blindly assessed by one pathologist using semiquantitative scores: steatosis (0=none, 1=<5%, 2=5-33%, 3=33-66%, 4=>66%); fibrosis (0=none, 1=perisinusoidal or periportal fibrosis, 2=perisinusoidal and periportal fibrosis, 3=bridging fibrosis, 4=cirrhosis); perisinusoidal fibrosis (0=none/mild, 1=moderate, 2=severe), lobular inflammation (1=<2 foci per 200x field, 2=2-4 foci per 200x field, 3=>4 foci per 200x field), portal inflammation (0=none to minimal, 1=greater than minimal), polymorphonuclear leukocytes infiltration (0=none to moderate, 1=severe), cholestasis (0=none, 1=mild, 2=moderate to severe), satellitosis (0=none, 1=a few, 2=many), ballooning hepatocytes (0=none or a few, 1=many) and Mallory bodies (0=none or a few, 1=many). Univariate and multivariable analyses were performed to identify histopathological parameters associated with MELD score, presence of severe AH (compatible histology, serum bilirubin > 5 mg/dl and mDF >32) and with 6-month survival.

Results: In univariate analysis, patients with increased polymorphonuclear leukocytes infiltration ($p=0.012$), cholestasis ($p<0.001$), satellitosis ($p=0.005$) and Mallory bodies ($p=0.008$) had a significantly higher MELD score. In addition, perisinusoidal fibrosis ($p<0.001$), lobular inflammation ($p=0.018$), cholestasis ($p<0.001$), polymorphonuclear leukocytes infiltration ($p=0.009$), satellitosis ($p<0.001$), ballooning hepatocytes ($p=0.008$) and Mallory bodies ($p<0.001$) were associated with severe AH (mDF>32). Furthermore, increased perisinusoidal fibrosis ($p<0.001$), polymorphonuclear leukocytes infiltration ($p=0.022$), satellitosis ($p=0.004$) and cholestasis ($p<0.001$) were associated with a greater 6-month mortality. In multivariable analysis, only cholestasis was independently associated with MELD score ($p<0.001$). Moreover, cholestasis and perisinusoidal fibrosis remained associated with severe AH ($p<0.001$ and $p=0.030$) just as with 6-month mortality ($p=0.011$ and $p=0.046$).

Conclusions: Typical histopathological features are associated with both severity and survival in AH. These histopathological parameters may contribute to identify patients with worse form of AH and poor prognosis.

O 06

Immunohistochemical staining of blood and lymph vessels in canine mammary tumours

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In women as in bitches, mammary tumours are the most common tumours. These tumours consist of tumour cells and tumour-associated stroma. An important component of the stroma is the tumour vasculature (blood and lymph vessels). Extensive research shows induction of angiogenesis and lymphangiogenesis in human breast cancer. These newly founded vessels facilitate tumour growth and metastasis.
In contrast to angiogenesis, no information on lymphangiogenesis in canine mammary tumours (CMTs) is available in literature. The main reason for this is the lack of reliable markers for immunohistochemistry (IHC) of lymph vessels in the dog. In human medicine, different markers for lymphatic endothelium are available: Prox-1, Lyve-1, podoplanin and D2-40. Recent research showed D2-40 to be the most suitable and reliable antibody to stain lymph vessels in human breast cancer. Dogs with spontaneous CMTs are considered a promising animal model for human breast cancer. If lymph vessels can be visualized on a reliable way, dogs with spontaneous CMTs can be harnessed as an animal model in comparative oncology trials with e.g. anti-lymphangiogenic therapies. Therefore, the possibility to stain canine tumour lymph vessels with human lymph vessel markers (Prox-1, Lyve-1, podoplanin and D2-40) was investigated in the present study. Ideally, these lymphatic markers are used together with blood vessel markers to discriminate between the two vessel types. Hence, staining of the blood vessels was performed with the blood vessel markers vWf, CD31 and CD34. A comparison was made between the different lymph vessel markers and between the different blood vessel markers to identify the most suitable lymph and blood vessel marker in CMTs.

Since IHC with antibodies against podoplanin, D2-40 and CD34 showed no immunoreactivity in CMTs, only Prox-1, Lyve-1, vWf and CD31 were retained. Tissue samples of five benign CMTs and five malignant CMTs were selected. For each sample, an H&E staining, an anti-Prox-1, an anti-Lyve-1, an anti-vWf and an anti-CD31 IHC staining were performed on serial sections. For each tumour, two intratumoural and two peritumoural areas with the highest vascular density (hotspots) were identified at low magnification. In these hotspots a comparative analysis (according to a variety of parameters) of the IHC of the blood and lymph vessels was performed at a high magnification (20x objective lens). Prox-1 and CD31 showed the best results after analysis.
In conclusion, human lymph vessel markers are able to stain lymph vessels in CMTs with Prox-1 being the marker of choice. Together with CD31, the best blood vessel marker, an evaluation of the lymphangiogenesis and angiogenesis in CMTs becomes possible. This facilitates the use of dogs with spontaneous CMTs as an animal model in comparative oncology studies e.g. in those in which anti-lymphangiogenic therapies are evaluated to decrease the metastatic potential.

O 07
SIGNIFICANCE OF INTRAOPERATIVE CONSULTATION IN INTRACRANIAL MENINGIOMAS

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OBJECTIVE: To investigate the current role of intraoperative cytological consultation in the management of intracranial meningothelial tumors.

STUDY DESIGN: The present study made a retrospective analysis of 62 consecutive patients with intracranial meningothelial tumors operated on during 01.01.2008-31.12.2009, in the Department of Neurosurgery, Emergency Clinical Hospital "Prof. dr. N. Oblu" Iaşi. Smears were prepared from the fresh biopsy samples sent to The Department of Neuropathology of the same hospital for immediate processing. The smears were stained with the 1% alcoholic toluidine blue method. Further, paraffin sections were prepared by the residual tissue and stained by Haematoxylin & Eosin method. The tumours were classified according to the World Health Organization classification of CNS neoplasms, 2007. Smear cytological diagnosis was correlated with histopathological findings and with demographic data of the patients. The observations were then subjected to appropriate statistical analysis methods.

RESULTS: Of the 62 patients, 41 were females (66.13%) and 21 were males (33.87%). 16 patients (25.80%) were in the age group of 41-50 years. There was concordance between intraoperative consultation and the final diagnosis in 58 cases (93,54%). A complete correlation was achieved in 51 cases (82.25%) and a partial correlation in 7 cases (11.29%) as there were underestimations of the malignancy grade. In four cases (6.45%), intra-operative consultation was discordant with final diagnosis because of the technical errors noted on smear slides such as intense tumoral vascularity, thickness of the smears and of difficulties of smears interpretation as cell morphology of glioblastomas resembles well with those of anaplasic meningiomas.

CONCLUSION: Squash smear technique in neurosurgery is a rapid and inexpensive method of intraoperative diagnosis and lead to a high diagnostic concordance.

O 08

IGF-IR and IGF-IIR/Man-6-P: new putative targets in glioblastoma ?

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Introduction. Glioblastoma (GBM) represents the most frequent malignant primary brain tumor in adults. Despite progress in surgery, radiotherapy and chemotherapy, the overall survival of patients with GBM remains extremely poor with a 5-year survival rate of 3.3%. It is imperative to better understand the molecular mechanisms involved in GBM pathogenesis in order to develop new targeted therapies. It is also important to discover new biomarkers associated with prognostic values. The insulin-like growth factor (IGF) system is a complex network and many studies support a role for this system in the regulation of GBM cell biology. In the present study, we decided to evaluate the expression of IGF system members in a large series of GBM to assess the prognostic value of these biomarkers.

Material and Methods. IGFI, IGFII, IGF-IR and IGF-IIR/Man-6-P expressions were evaluated by means of quantitative immunohistochemistry in tissue microarray sections carried out from 218 human GBM and in 10 normal adult brain samples. The relations between expression of these IGF system members and various clinicopathological parameters were analysed. Further, *in vitro* experiments were carried out in order to characterize the IGF-IIR/Man-6-P role in GBM angiogenesis.

Results. In normal adult brain, we didn't observe significant expression of the IGF system members. The majority of GBM showed no expression of IGFI (75%) or IGFII (78%). In contrast, 64% and 52% of GBM cases showed IGF-IR and IGF-IIR/Man-6-P expression, respectively. Both IGF-IR and IGF-IIR/Man-6-P expressions were observed in the cytoplasm of tumor cells. Moreover, IGF-IIR/Man-6-P exhibited a dot-staining pattern in the cytoplasm of endothelial cells in hyperplastic vessels. As compared to normal adult brain, only IGF-IR and IGF-IIR/Man-6-P expressions significantly increase in GBM ($p<10^{-4}$ and $p=0.002$, respectively). Only IGF-IR expression exhibited a significant impact on the outcome of patients and was negatively associated with cancer-specific survival ($p=0.046$) in univariate analysis. Multivariate analysis confirmed an independent prognostic value for IGF-IR expression. Finally, preliminary *in vitro* experiments suggest that IGFII required the IGF-IIR/Man-6-P to promote tumor angiogenesis.

Conclusion. This study indicates that IGF-IR expression has prognostic value for patients with GBM. Moreover, our data propose an involvement of IGF-IIR/Man-6-P in angiogenesis. Together, these data suggest that IGF-IR and IGF-IIR/Man-6-P could be interesting targets for GBM therapies.

O 09

UROTHELIAL IN SITU CARCINOMA IN CONVENTIONAL CYTOLOGY

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Introduction

The primary application of urinary cytology is to detect high-grade urothelial tumors or carcinoma in situ and to monitor patients with a former history of urothelial precancerous or malignant lesions. Cytology has an adventage of sampling the entire urothelium and may be more sensitive than many biopsies in predicting tumor recurrence or progression.

Material and methods

A total of 43.632 voided urine specimens from 14.544 patients were collected from 2003 to 2011. for conventional cytology analyses. Urine specimens were collected in three consecutive day samples. Approximately 50 mL used to obtain cytospin centrifugation (Shandon Cytospin 4) resulting in 2 slide preparations per sample that were stained according to MGG.

Cytological picture shows mostly clean background and decrease of inflammation and necrosis, tumor cells were abundant, single or more often in small and non-papillary clusters. Cells are pleomorphic and bizzar, sometimes with moderately abundant cytoplasm. Nucleus are larger in size, with high N/C ratio, coarsely granular and hyperchromatic chromatin, irregular nuclear membrane and inconspicious nucleoli. Cytoplasm is often amphophilic.

Results

Cytological diagnosis suspicious on carcinoma in situ was obtained in 24 patients (22 men and 2 woman). Histopathological diagnosis was available in 20 cases, of which 15 confirmed urothelial carcinoma, one was suspicious for carcinoma, 3 were without malignant cells and in one case, the histopathological diagnosis was urothelial carcinoma in situ.

Conclusion

Cytology alone is insufficient for diagnosis of urothelial carcinoma in situ. The cytology and histopathology is hard to correlate, because of different collected sample. Furthermore, urothelial carcinoma in situ is a flat lesion, which is sometimes hard to visualisate on cystoscopy. This paper shows that carcinoma in situ exists with low and high grade transitional cell carcinomas, so patients with cytological diagnosis of urothelial in situ carcinoma must be treated like others with invasive urothelial carcinoma.

O 10

Diagnostic Value of the *UCA1* Test for Detection and Surveillance of Urothelial Cancer

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Introduction. Urothelial cancer detection and surveillance are traditionally performed by cystoscopy and urine cytology. Since both tests had proved insufficient in some cases, many non-invasive urinary tests were developed in order to overcome those drawbacks. Recently, *Urothelial Carcinoma Associated 1 (UCA1)* was identified as being a very sensitive and specific urine marker of urothelial cancer. This study aims to compare the clinical value of the *UCA1* test with routine diagnostic methods.

Methods. A prospective study of 264 patients was conducted at the Erasme University Hospital. Fresh voided urine samples were obtained from each patient for cytological examination and *UCA1* mRNA detection in urine sediment. Histological diagnosis was available for 73 patients. Taking the latters as the gold standard, we evaluated the sensitivity, the specificity and the positive predictive value (PPV) of the *UCA1* test in comparison with cystoscopy and cytology. The *UCA1* test results were statistically related to various clinicopathological parameters. The technical and the cytological features were finally studied in order to characterize the cases that were inadequate for evaluation.

Results. The sensitivity, the specificity and the PPV for the *UCA1* test were respectively 61.7%, 69.2% and 78.4%. Within the group of patients with a negative cytology result, the PPV of the *UCA1* test was 67%. The *UCA1* test results were not related to any clinical, histological and cytological feature under study. Cases inadequate for evaluation were mainly due to the poor cellularity of the urine samples.

Conclusion. The overall sensitivity and specificity of the *UCA1* test for the detection and surveillance of urothelial cancer in our study were lower than data previously reported. While cystoscopy remains the most sensitive diagnostic tool, further attention should be given to patients with negative urine cytology and positive *UCA1* test, a condition associated with a 67% incidence of malignancy.

POSTERS

POSTERS LIST

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P 01

How to integrate molecular testing in gliomas evaluation?

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Gliomas are the most frequent primary brain tumors and include a variety of different histologic types and malignancy grades, each associated with different prognosis. Histologic evaluation is the gold standard for the diagnostic of these tumors. However, diagnostic difficulties may arise from tumor heterogeneity, overlapping morphologic features and tumor sampling. Fortunately, abundant research has identified specific molecular markers that are characteristic of gliomas, according to diagnostic classification and tumor grade. Some of these markers have diagnostic value, whereas others are useful prognosticators for patient survival and therapeutic response.
Among them, the combined loss of the short arm of chromosome 1 (1p) along with of the long arm of chromosome 19 (19q) is a diagnostic marker of oligodendroglial tumors. Moreover the 1p19q codeletion is also associated with better prognosis and higher sensitivity to chemotherapy.
The detection of mutations in Isocitrate deshydrogenase 1 (IDH1) gene can be of help in the distinction between diffuse astrocytoma (grade II) and pilocytic astrocytoma (grade I) and in the differential diagnosis of primary and secondary glioblastoma. In addition, IDH1 mutation status is also of great prognostic relevance. Hypermethylation of the O^6-methylguanine-DNA methyltransferase (MGMT) gene promoter is a predictive marker for the response to alkylating agents such as temozolomide in high grade astrocytomas. Finally, the deletion of the CDKN2A gene (p16) and the amplification of the epidermal growth factor receptor (EGFR) can be used as diagnostic markers of high grade astrocytomas.
In our lab we choose to develop, under the ISO15189 standard, molecular tests which are now integrated in the routine assessment of gliomas as diagnostic, prognostic and predictive markers. **For practical purposes we have established an algorithm for judicious use of these molecular testing in diagnostic and prognostic approach. This is of great help to enhance the quality and the relevance of information provided to clinicians**.

P 02

Implementation of a computer system of traceability: experience of the Erasme Hospital Pathology Department.

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Introduction
As most pathology departments in Belgium, the Erasme Hospital pathology department is committed to a quality approach and is accredited according to the NF EN ISO 15189 standard. The quality policy requires a continuous improvement of the services provided by the laboratory. In this context, we noticed that an IT management system should not only allow the creation of reports but also insure an optimal traceability of the diagnostic chain, from the reception of samples to the report of the diagnosis to the clinicians.

Materials and methods
The pathology department manages its samples and reports by means of the software Diamic (Infologic-Santé). In order to establish a correct traceability within the laboratory, we have developed in association with Infologic-Santé, a module of traceability integrated to Diamic.
An audit was performed by Infologic in order to define the steps to track, the adjustments to make to the configuration of the software as well as the needs in equipment.
Implementation of the traceability module was divided in four phases: 1) general update of the software in the adequate version and training, 2) installation of the module, training and development, 3) use of the module and request of improvements, 4) optimization of the module.

Results
We report the different improvements allowed by the implementation of the module, as well as its limitations and disadvantages. Limitations mostly relates to IT, such as for instance connection with immunohistochemistry software. The major disadvantages are: time consuming procedure, difficulties in involvement of the staff and disagreements on modifications of working procedures. The positive points are: empowerment of the staff, better legibility, better control over pre-examination procedures and strengthening of the team spirit.
A catalog of “requests” is currently available and allows obtaining detailed statistical data, including quality indicators such as “fixation time”. The aim is to obtain a complete technical history for each samples with at any time all the informations concerning the technical steps.

Conclusion
The traceability module developed in association with Infologic-Santé mainly meets the needs of Erasme Hospital pathology department. It is however necessary to underline that the implementation of such a system of traceability generated a significant financial cost, linked to the development of the traceability module and the acquisition of additional equipment. Additionally, consequent changes in the working habits of the entire staff of the laboratory were required to adapt to this new traceability system.

P 03

Unusual adrenal cytological diagnosis.

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In the assessment of a 68 year old patient with a pancreatic mass, the CTscan revealed an fatty adrenal mass. The FNA performed under EBUS showed normal hematopoietic cells.
Only cytology bring the definitive diagnosis of myelolipoma.

The authors present the diagnostic keys of this unusual but caracteristic cytological picture and review of the literature.

Myelolipoma may yield adrenal masses observed at medical imagery and characterized microscopically by the presence of normal hematopoietic cells.

To correctly diagnose this unusual pathology the cytopathologist must:

1. known the clinical and imaging datas
2. identify in the FNA product a normal hematopoietic cell population
3. known the nomenclature –the list- of the possible diagnoses of adrenal masses
4. identify extramedullary hematopoiesis of the adrenal gland

To plagiarize Einstein, we dare to say : « Pathologist can only diagnose lesions he/she know - at least theoretically. ».

P 04

A gliosarcoma with synchronous supra-infratentoriallocalizations: a case report.
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INTRODUCTION
Gliosarcomais rare neoplasm of the central nervous system with a poor prognosis. It isaglioblastoma variant,characterized by a biphasic tissue pattern with alternating areas displaying glial and mesenchymal differentiation. Gliosarcoma affects adults in the sixth to seven decade of life having a male predominance. Temporal lobe is involved in themost of the cases. Metastases from gliosarcoma are infrequent and usually in extracranial sites but not in the central nervous system.To our knowledge, only three cases of gliosarcomahave been reported with multiple intracerebral locations (1,2,3).
CASE REPORT
Our patient is a 45-year-old Caucasian man that developed vertigo, hypoacousia, hypoesthesia of the right hemiface and intermittent vertical diplopia. CT Scan and MRI revealed two tumoral masses:one in the right frontal lobe (2 cm of diameter) and the other one in the right cerebellar peduncle (3.5x2.5x2.5 cm). Three additional millimetric lesions, suspect of being tumoral, were also seen: two in the right frontal lobe and one in the corpus callosum. Based on the radiologic findings, a diagnosis of metastases was suggested. A metastatic work-up was negative. The patient underwent a suboccipital craniotomy and the right cerebellar pedunclemass was partially removed.

Microscopic examination revealed a biphasic tumor with alternating areas of gliomatous and sarcomatous differentiation. The glial component was strongly positive for GFAP. The sarcomatous tumor cells were negative for GFAP and positive for Vimentin. A dense reticulin network was identified in the sarcomatous area. A high expression of p53 was observed in both tumoral patterns.R132H-IDH1 immunohistochemistry was negative. The proliferation index was evaluated at 30%. A diagnosis of gliosarcoma was performed.

CONCLUSIONS
We described anunusual case of gliosarcoma showing synchronous supra-and infratentorial localization. This combinedoccurrence has been described in gliomas, but is rarer then multiple supra-tentorial locations. It raises the issue for such gliomas to be multifocal or multicentric. The former is favor when an anatomical continuity is recognizable in between masses as cerebrospinal fluid, commissural fibers or basement membranes of blood vessels. The latter is otherwise suggested (4). In the current case, pyramidal pathway links the two main tumors, and a millimetric masse is observed in the corpus callosum. This suggests the present gliosarcoma to be multifocal, an unusual finding in a tumor classically associated with extra-central nervous system metastases when they occur.

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P 05

HEPATOCELLULAR ADENOMAS AND LIVER VASCULAR ABNORMALITIES

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INTRODUCTION: Hepatocellular adenomas (HCA) are rare benign liver tumors known to be more frequent in women on oral contraceptives. They can also occur in men on anabolic steroids or in some metabolic diseases.

AIM: The recent observation in our routine practice of an association between portal vein agenesis and adenoma prompted us to evaluate the presence of vascular abnormalities in our series of HCA and to study the characteristics of the lesions.

MATERIEL AND METHODS: We reviewed our series of 37 surgically treated adenomas and identified 3 cases associating hepatocellular adenomas and portal venous anomalies. Their clinical history, as well as radiological images, and pathological results were analyzed.

RESULTS: The patients included a male child of 11 yrs, a 42 year-old man and a 68 year-old female. The young child suffered from Klippel-Trenaunay syndrome associated with an agenesis of the portal vein and was followed since 10 years for multiples hepatic adenomas. Because of worsening of liver imaging and rising alpha-foeto-protein levels, he underwent liver transplantation. The surgical specimen contained a single mass of approximately 17 cm in its greatest dimension, occupying almost the entire right lobe, consisting in a Beta-catenin mutated adenoma with areas of transformation into hepatocellular carcinoma (HCC) a and numerous vascular invasions. The adult male presented with asthenia and microscopic hematuria warranted an ECHO and CT abdomen, with the surprising discovery of a dysmorphic left liver lobe, containing multiple lesions, confirmed on MRI to correspond to adenomas and absence of the left portal vein. The surgical specimen, revealed a large mass of 18 cm consisting again in a Beta-catenin mutated adenoma with areas of transformation into HCC. The adult female was also diagnosed by chance with multiple adenomas in the left lobe of the liver while being checked up by a CT scan for renal stones. Severe steatosis as well as left portal vein atrophy was also observed. In her past history she was treated for breast cancer. The surgical specimen demonstrated the presence of numerous adenomatous nodules (> 10), of which the largest one measured 8 cm. Macrovacuolar steatosis was observed in all lesions. Immunohistochemistry revealed a negative staining for L-FABP indicating a probable *hnf1-alpha* mutated adenoma. No transformation into HCC was noted.

CONCLUSIONS: Do vascular abnormalities of the liver predispose to HCA? An association between congenital vascular liver abnormalities and benign liver neoplasms such as hemangiomas, focal nodular hyperplasia, nodular regenerative hyperplasia and HCA as well as hepatoblastoma and hepatocellular carcinoma has been reported in the literature. The proposed pathogenesis includes uneven perfusion of the liver responsible for the development of various types of nodular lesions the characterization of which is not always straightforward.

P 06

Aortopulmonary fistula in Friesian horses

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Introduction:
Aortopulmonary fistulation is very rare in animals. In Friesian horses however, this condition is more frequently encountered.
In humans, acquired aortopulmonary fistulas are rarely reported and often only diagnosed post-mortem. They occur most frequently as the result of erosion and/or rupture of a degenerative or false aneurysm and are probably caused by continuous pulsatile friction. An investigation of 4000 autopsies involving thoracic aorta aneurysms revealed that only 3.7% ruptured to the pulmonary artery. Aortopulmonary fistulation has been associated with trauma, inflammation, arteriosclerosis, aortic surgery, lupus and Marfan's syndrome. Less frequently they are found in the progression of acute aortic dissections.

Materials and methods:
Ten Friesian horses were submitted for necropsy. There was a suspicion of aortopulmonary fistulation based on history, clinical signs, echocardiography and cardiac catheterisation. A complete necropsy, as well as histological examination of the aortic wall was performed.

Results:
In all cases, a rupture of the aortic arch near the ligamentum arteriosum with fistulation into the pulmonary artery was present. Several extensive perivascular hematomas were observed that communicated with both arteries and are considered pseudoaneurysms. Histological examination of the aortic wall adjacent to the rupture revealed thickening of the media by fibrosis, increase of mucinous stroma and elastin fragmentation. A mild infiltration of inflammatory cells was found.

Conclusion:
Unlike some human cases, no history of chest trauma or thoracic surgery was present and histological examination revealed no signs of infection. Due to the typical and unique location of the aortic rupture in all cases, a hereditary origin of the condition is suggested.

P 07

Atlas of Genetics and Cytogenetics in Oncology and Haematology
Peer reviewed Internet Encyclopedia/Journal/Database:http://AtlasGeneticsOncology.org

Jean Loup Huret, Mohammad Ahmad, Mélanie Arsaban, Alain Bernheim, Maureen Labarussias, Vanessa Leberre, Anne Malo, Franck Viguié, Alain Zasadzinski, Philippe Dessen

Issue: Prognosis of a leukaemia depends on the genes involved: median survival of 3 months in case of inv(3) versus 95% patients cured in the case of a dic(9;12). Treatments depend on the gravity of the disease. However, while some diseases are very frequent, others may be very rare (1 published case), in particular in cytogenetic subsets of leukaemias ... and there are more than 750 subtypes of leukaemias! 2 000 to 9 000 genes are possibly implicated in cancer, and 25 000 new publications concerning cancer genetics in man were added every year to PubMed. If one wants to save as many patients as possible, it is therefore essential to have this necessary knowledge at disposal.

Hypothesis: Thus, there was a need for a huge database collecting and summarising the fast growing knowledge accumulated in cancer genetics.

State of the art: The Atlas is a peer-reviewed internet journal / encyclopaedia / database aimed at genes involved in cancer, cytogenetics and clinical entities in cancer, and cancer-prone diseases. It is a collective effort from researchers and clinicians to give the state of the art in cancer genetics to the medical and scientific community, to provide a cognitive tool for fundamental and clinical research. Readers of the Atlas are consultants at the hospital, researchers, university teachers, but it also reaches students in medicine and students in sciences.

Objectives: The Atlas is part of the genome project and it participates in research on cancer epidemiology. It is at the crossroads of research, university and post-university teaching (virtual medical university) and telemedicine. It contributes to 'meta-medicine', this mediation between the overflowing information provided by the scientific community and the individual practitioner. It helps the cytogeneticist in his diagnosis, it helps the clinician in therapy decision. The Atlas is in free access, including for the third-world countries.

Specificity of the Atlas The Atlas brings together and combines various knowledge in one site: genes and their function - cell biology, diseases and clinical implications, cytogenetics, indeed, and also clinical genetics: hereditary diseases with an increased risk of cancer. This unifies cancer genetics, while data are dispersed in various and heterogeneous sites elsewhere. The iconography is diverse (ultrasounds, pathology, chromosomes, 3-D structure of proteins, gene maps...) and abundant (more than 11 000 images). It is the only one site devoted to genetics where prognosis is given.

Planned schedule

- In a year, the Atlas has accepted and published more than 230 papers. The Atlas now reaches 35 000 pages with 2 100 authors. ... and we need more authors !

- 5000 machines connect every day, with 1 000 000 visits a year (25% from the USA, 3 700 from Brussels, and about 1 000 visitors/laboratoires coming more than once a week).

- We wish to be indexed in PubMed. We are therefore developing an 'electronic journal' version of the Atlas, comprising 85 volumes from 1997 to 2012. There is about 2 200 papers included in the journal, making 7 000 pages of peer reviewed articles in pdf format.

We wish scientific Societies to be implicated in the Atlas develpment: Cytogeneticists indeed (Association des Cytogénéticiens de Langue Française, European Cytogeneticists Association), Geneticists in general, but also Pathologists; Belgian Societies of Pathology are welcome!

P 09

Superior aero-digestive tract dysplasia: case report and discussion of the current classification

Sebastien Duquenne (1), Pierre Demez (1), Albert Thiry (1), Philippe Delvenne(1), Sven Saussez (2) / [1]CHU Sart-Tilman, Liege, [2]Anatomy and cellular biology laboratory, Mons

We report the case of a 78 year-old woman with an important history of oral neoplasias who received consultation for two lingual lesions. The first one was located on the left-hand side of the tongue and measured 2.5 cm. It was firm and exophytic. The second one, located on the right-hand side of the tongue, was small (0.6 cm), poorly demarcated in a flat whitish area. Biopsies led to the diagnosis of verrucous carcinoma and micro-invasive carcinoma respectively on the left and right sides. After resection, the left-hand lesion was diagnosed as a well-differentiated squamous cell carcinoma, NOS, whereas the right lesion did not display any stromal invasion after resection, and was diagnosed as a low grade intra-epithelial epidermoïd neoplasia (mild dysplasia).
Important discrepancies between pre-operative biopsies and final diagnosis give the opportunity to review dysplasia's criteria and to discuss recent data on this matter. Several authors has proposed different classifications during these last 30 years, which have been used until a consensus was obtained in the 2005 WHO classification. This classification has the advantage to lower inter-observer variability, and to bring some coherence in this hard-debated subject. However, some argue that it does not take into account all the elements likely to be relevant in the appreciation of the progression risk to invasive squamous cell carcinoma. The WHO classification is based on a three grade system corresponding mainly to the height reached by the epithelial atypia and architectural disorganization. Earlier propositions by Kambic et al (1997), and later by Hellquist (1999) based on the Ljubjana consensus also include the importance of atypias and the more subjective but very important notion of "dysmaturation" or forward maturation in the bottom of the epithelium.
Other interesting topics in this discussion are the usefulness of immunohistochemistry, particulary P16, P53 and Ki67, which can be helpful but not very discriminant. A recent research also emphasizes new potential markers, such as Maspin, which could help to differentiate lesions susceptible to progress to squamous cell carcinoma.
In summary, the goal of these modifications is to better evaluate the risk of progression, but new breaktroughs are needed in this field to better help clinicians to distinguish low risk lesions from high risk ones.

P 11

Systematic review of Gestational Trophoblastic Diseases by an expert panel of pathologists

Timmermans M (1) , Delbecque K **(1)** , Delvenne P (1) , Marbaix E (2) , Noël JC (3) , Moerman P [5] , Vergote I[5], Kridelka F (1), Nisolle M (1), Goffin F (1), On behalf of the GTD.be group / [1] University of Liège, [2] UCL St-Luc, [3] ULB Erasme, [4] Catholic University of Leuven,

Introduction

Gestational trophoblastic diseases (GTD) represent a spectrum of pregnancy-related lesions (complete and partial mole) with potential invasive or malignant behaviour (invasive mole, choriocarcinoma and placental site trophoblastic tumour). In Western populations, the incidence of complete and partial molar pregnancy is 1 and 3 per 1000 births, respectively. In Belgium, 100 complete moles and 10 choriocarcinomas are expected per year.

The low incidence makes these lesions relatively unknown. Subsequently, the management is shown to be inadequate in a high proportion of cases. According to the French experience, 30% of these cases are over or under treated, due to misdiagnosis, short follow-up or inadequate chemotherapy regimen.

During the last World Congress on GTD, in October 2011, many countries (France, UK, The Netherlands, Switzerland, US,…) shared their experiences about treatments and follow-up. The benefit of the centralisation for the management of GTD was clearly shown.

Aim

The aim of this project is to organize a GTD registry in Belgium to improve the pathological diagnosis, follow-up and treatment of these rare diseases.

Materials and methods

A web-based registry (www.BGOG.eu) and two reference centers have been set up, with the support of the Flemish and French-speaking gynecological societies (VVOG and GGOLFB) and the European Organisation for the Treatment of Trophoblastic Disease (EOTTD). The reference center's work is based on a multidisciplinary approach between pathologists, general and oncological gynecologists, radiologists and medical oncologists, all working to determine the right diagnosis and to define adequate follow-up, staging, scoring and treatments.

In practice, once a molar pregnancy is diagnosed,

- the center is contacted by the treating physician to register the patient,
- signed informed consent is obtained ,
- register form is completed by the treating physician,
- a review of the pathology is requested,
- the referring pathologist submits the case to the expert panel of pathologists (*),
- the hCG regression curve is plotted,

In case of gestational trophoblastic neoplasia, a written counselling is provided by the reference center to the referring physician in concordance with the FIGO/WHO and Belgian GTD guidelines. During all the process, the patient remains treated by her

physician.
Data are encoded in a secured web-database, with restricted access to the centers.

Conclusion

This Belgian initiative, encouraged by the European network of GTD, is set up to improve health care for GTD patients. The cornerstones of this organization are the systematic pathological review by an expert panel of pathologists (*) and clinical advice by the reference centers.

P 12

Catteau (1), Noël (1), Dargent (2), Dehou (3)/ [1] UMB Erasme, [2] Genetic and Pathological Institute, Gosselies [3] CMP laboratory, Brussels

Introduction.
Secondary localization of chronic lymphocytic leukemia (CLL) in breast is rare, while concurrent invasive ductal carcinoma and CLL manifesting as a collision tumor in breast is extremely rare.

Materials and methods.
Hereafter, we report a second case of an 80 year-old woman in whom a leukemic infiltrate was confined to the region immediately surrounding poorly differentiated primary breast carcinoma.

Discussion.
This association, CLL and carcinoma, has also been described in other organs, and particularly skin. The presence of both tumors is not simply due to chance and several hypothesis are proposed to explain this association :

1) A preferential recruitment of neoplastic cells at sites of immune reactions because of the predominant neoplastic composition of the lymphocytic pool in CLL patients.
2) A paracrine action on leukemic cells at the site of carcinoma-associated neovascularization.
3) The CCL cells are recruited at the medullary carcinoma interface with the help of membranous factors (intercellular adhesion molecules), as well as of soluble ones (cytokines).
4) An underlying etiology predisposes both tumor types (mutation ATM gene or infection by Epstein-Barr virus).
5) The CLL may express a B-cell receptor with affinity for an undefined breast cancer antigen.

Conclusion.
The observation of a CLL infiltrate closely associated with a distinct breast neoplasm in the absence of any other localization for the leukemia is indeed an indisputable argument for a relationship between the two diseases. Further supplemental studies are needed to confirm this association.

P 13

Asymmetrical campomelic dysplasia-like syndromeof unknown etiology in a colony of Common Marmosets (*Callithrixjacchus*)

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In a closed colony of Common Marmosets, bred for research purposes, several adolescentand adult animalspresented with abnormal bowingof the long bones. Despite its severity, this malformation was only observed during the yearly clinical check-up, since affected animalsshowed no pain, discomfortor altered behavior, but only displayed the inability to entirely stretch their lower limbs.

To further characterize this condition phenotypically, a group (n=57) of animalswas clinically examined. Forty-one of those marmosets were euthanized for unrelated reasons and examined macroscopically. Of half of those animals, the bones were examined after maceration of the skeleton. Theremaining16 living animals were monitored clinically on a daily basis and by radiography on a monthly basis. The data were compared with age-matched controls.

One of the 16 living animals showed severe progressivediaphysealbowing of the humerus, radius-ulna, femur and tibia-fibula from the age of 9 months onwards. Once the growth plates of the bones had closed, no further pathological progress was seen. Of the 41euthanized animals, seven showed similar abnormalities. In everyindividual, not all bones are affected equally, resulting in asymmetry of the condition. No other skeletal deformities or clinical illnesses were present in affected animals.A familiar predisposition could not be found based on pedigree analysis of the affected animals.

Based on the clinical and macroscopic observations, the condition was diagnosed as an asymmetrical campomelic dysplasia-like syndrome.The differential diagnosis of this unknown syndrome includes different metabolic bone diseases and growth dysplasias. However, since no other clinical abnormalities were detected and since further bowing of the bones arrestswhen the growth plates close,a congenital growth disorder seems more likely. Only one similar case has previously been reportedin a rhesus macaque (*Macacamulatta*). In human medicine, no exactly matching dysplasia is known, although it most resembles the skeletal deformities seen in dyschondrosteosis caused by a mutation in the SHOX-gene. Currently, blood of affected and non-affected animals is being examined for hepatic and renal markers, vitamin D and parathyroid hormone.

P 14

Type 1 CCAM affecting the entire left lung associated with a hypoplasia of the left heart.

Lelotte Julie (BSC), Camboni Alessandra (CHU Saint-Luc, Woluwé, Brussels), Wallon Joëlle (CHU Saint-Luc, Woluwé, Brussels), Godfraind Catherine (CHU Saint-Luc, Woluwé, Brussels)

Congenital cystic adenomatoid malformations (CCAM) are rare lung malformations affecting approximately one birth in 25 – 35,000. They arise between the 5th and 16th week of gestation, during the pseudoglandular stage of the lung development. We can classify them in three types according to the classification of Stocker, the latter being based on criteria such as macroscopic and histological. They can be associated with other malformations, in particular cardiac or renal. The forecast of these lesions depends mainly on the size, type, prenatal complications (such as a hydrops) as well as associated malformations.
We are reporting the case of a fetus having undergone a termination of pregnancy at 21 weeks of pregnancy for a voluminous left thoracic mass for which no precise diagnosis had been able to be given. During the autopsy, this mass turned out to be a type 1 CCAM according to the classification of Stocker, consisting essentially of a large-sized cyst. The latter affected the entire left lung and was associated with a major hypoplasia of the left heart.
It is so an unusual case of type 1 CCAM affecting a complete impairment of the left lung as well as a left cardiac hypoplasia. This association is, to our knowledge, the first case to be reported.
From this case, we have realized a review of all the congenital lung lesions listed in our department and have compared these to the literature.

P 15

Congenital ascites due to hepatoblastoma with peritoneal implantation metastases in an equine fetus

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Ghent University

An 11-years-old primiparous Belgian Warmblood mare suddenly showed enlargement of the udder, premature galactorrhea and vaginal discharge at 317 days of gestation. Vaginal exploration revealed the presence of a dead foal in a cranial dorsosacral position with a ventral deviation of the head. Even after reposition of the head, extreme force was necessary to extract the foal. Gross examination of the dysmature filly showed an abnormal and excessive fluctuating distension of the abdomen and yellow mucosae and the fetus was submitted to a full post mortem examination.

Necropsy revealed an irregular bulging, nodular, solitary, firm, yellow mass of 25 cm in diameter in the right liver lobe. Multifocal to coalescing metastases, ranging from 0.5 to 3 cm in diameter, were present in the left liver lobe, on the peritoneum and on the serosal surfaces of the stomach, the diaphragm, the spleen and the intestine. There was approximately 1 liter dark, turbid, red fluid in the abdominal cavity.

Histopathology of the hepatic mass revealed a well-demarcated, encapsulated, expansile, densely cellular neoplasm, which consisted of embryonal epithelial cells. The tumor cells were polygonal, 15-18 µm in diameter, with indistinct cell borders, arranged in sheets and nests. The cells had scant, pale eosinophilic cytoplasm and a single, round to oval, central, basophilic nucleus, with finely stippled normochromatic chromatin were present. Some nuclei contained a single basophilic nucleolus. There was mild anisocytosis and anisokaryosis. Mitotic figures were rare. The cells were supported by a loosely arranged fibrous stroma. All metastases were composed of embryonal epithelial cells arranged in sheets and nests and were similar to the tumor cells of the primary tumor. All other tissues revealed no abnormalities.

Immunohistochemically, the neoplastic cells stained diffusely positive for alpha-feto protein (AFP) and multifocally positive for pancytokeratin and Low-Molecular-Weight cytokeratin. Ki67 staining was also multifocally positive, with approximately 30 positive cells per high power field. The mesenchymal cells and fibrous stroma cells stained positive for vimentin. The High-Molecular-Weight cytokeratin, CD3, CD20, synaptophysin, alpha-1-antitrypsin, neuron-specific enolase, S-100 and E-cadherin stainings were negative.

Based on histopathology and immunohistochemistry the diagnosis of an embryonal type hepatoblastoma was made. Although hepatoblastoma is the most common primary malignant hepatic tumor in human infants (Hadzic and Finegold, Clinical Liver Disease, volume 15, p. 443-462), only 11 equine cases have been described (Beeler-Marfisi *et al.,* Journal of Veterinary Diagnostic Investigation, volume 22, p. 174-183.). To the author's knowledge, no other reports of ascites due to hepatoblastoma with peritoneal implantation metastases have been described in an equine fetus.

P 16

Rheumatoid leptomeningitis: a rare complication of rheumatoid arthritis

Hastir D., D'Haene N., Salmon I., Maris C. / ULB Erasme

Rheumatoid leptomeningitis is a rare but one of the most severe complications of rheumatoid arthritis (RA). The mortality rate of rheumatoid leptomeningitis is relatively high and diagnosis can be difficult.

We describe a 63-year-old woman with a RA history admitted with apathy, drowsiness, fatigue and difficulty concentrating over a three-week period. Magnetic resonance imaging revealed hyperintense lesions in both frontal lobes on T2-weighted, fluid-attenuated inversion recovery (FLAIR) and diffusion-weighted images with an abnormal contrast enhancement in leptomeninges and cortical bands. ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET) revealed high glucose uptake in those areas. The proposed diagnosis was meningo-encephalitis. Cerebrospinal fluid analysis showed a reactive process with presence of numerous lymphocytes and plasmocytes. Biopsies of brain tissue and dura mater from right frontal lobe were obtained. Histopathological analysis revealed necrotizing granulomas, lymphoplasmocytic infiltrates and giant cells involving the leptomeninges, consistent with rheumatoid leptomeningitis. This diagnosis should be based on the exclusion of diseases that may cause similar granulomatous reactions including infectious disease such as tuberculous meningitis. Despite aggressive treatment including corticosteroids and immunosuppressive agents, the patient died three months later.

In conclusion, this case illustrates a rare but very aggressive complication of RA.

P 17

Detection of IDH1 mutations in human gliomas : is immunohistochemistry useful ?

D'Haene N., Maris C., De Nève N., Allard J., Verrellen A., Le Mercier M., Salmon I./ ULB Erasme.

Introduction: Hotspot mutations in codon 132 of the gene encoding isocitrate deshydrogenase 1 (IDH1) are a frequent genetic alteration in astrocytomas, oligodendrogliomas, oligoastrocytomas and secondary glioblastomas. IDH1 mutations are associated with a favorable patient survival. Given the diagnostic and prognostic implications of IDH1 mutations, all gliomas should be assessed for IDH1 mutation status in a near future. Most published studies to date analyzed the IDH1 mutation by gene sequencing. Different methods of detecting IDH1 mutation have been described, including polymerase chain reaction (PCR) - and restriction endonuclease-based method, detecting all mutations in IDH1 codon 132 and immunohistochemistry (IHC).
Materials and methods: In order to assess the value of the specific monoclonal antibody, H09, which detects R132H IDH1, the most frequent IDH1 mutation, we compared the results of IHC and the results of PCR- and restriction endonuclease-based method in formalin-fixed and paraffin-embedded samples of 66 diffuse gliomas. Among these cases, 38 cases were also analyzed by gene sequencing.
Results: *1. IHC results.* Twenty-two cases were evaluated as IHC positive of which 21 were characterized by a strong cytoplasmic staining within a majority of tumor cells. One case was characterized by foci of positive infiltrating tumor cells. *2. IHC/PCR- and restriction endonuclease-based method comparison.* All the 21 IHC strongly positive cases carried IDH1 mutation. In the case with foci of positive cells, the PCR analysis was inconclusive. Among the 44 IHC negative samples, 38 were IDH1 wild-type and 6 showed a IDH1 mutation. *3. IHC/gene sequencing comparison.* Among the 38 cases evaluated by gene sequencing, we observed 10 IHC strongly positive samples, all with a R132H IDH1 mutation. The 27 IHC negative cases were determined to be wild type for 24 and to harbor a non-R132H IDH1 mutation for 3. The case with foci of IHC positive cells (described in point 2) was also inconclusive by gene sequencing. *4. Value of IHC.* Given that all IDH1 codon 132 mutations are clinically relevant, we compared the results of IHC with the results of PCR- and restriction endonuclease-based method, leading to sensitivity and specificity of IHC to detect all types of IDH1 codon 132 mutations of 77.8% and 100% respectively. For a single case with foci of IHC positive cells both genetic testing methods were inconclusive, leading to the hypothesis that tumor cell content was below the sensitivity limit of the genetic methods.
Conclusion: This study confirms IHC as a reliable method for detection of R132H IDH1 mutation. However, negative cases should be tested by other genetic methods to exclude variant mutations. Moreover, in some samples IHC could be more sensitive than genetic methods to detect positive infiltrating tumor cells.

P 18

SAFETY ASSESSMENT IN PIGS OF AN EXPERIMENTAL MOLECULE WITH IN VITRO ANTIVIRAL ACTIVITY AGAINST AFRICAN SWINE FEVER

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Introduction: Several nucleoside and nucleotide analogues have been identified and approved as potent antiviral agents. HPMPDAP (*9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine*) belongs to the group of acyclic nucleoside phosphonates (ANPs)that have proven their efficiency with convincing activity against poxviruses, cytomegalovirus retinitis in AIDS patients, chronic hepatitis B virus infections, and human immunodeficiency virus infections. African swine fever virus is the causative agent of African swine fever and can cause devastating outbreaks in pigs, with important economic impact. The disease is endemic in most of the African countries and since 2007 has spread dramatically from the Caucasus region to neighbor countries including Russian Federation. Up to now conventional approaches to develop a vaccine have remains unsuccessful and control strategies to prevent spread of the virus are limited to prophylactic measures and pre-emptive culling. In this context, antiviral approach could provide an alternative solution in epidemic situations. In order to evaluate if HPMPDAP could also be used against CSFV infection, a toxicity study was performed in domestic pigs.

Materials and Methods: Four groups of two large white pigs were intramuscularly injected with 4 different doses of the molecule. There was daily clinical observation and blood collection. The blood parameters that were controlled included hematology, ionograms, renal and liver parameters. Autopsy was performed 3 to 4 days after the last administration. Samples of liver and kidneys were taken for further histopathological examination.

Results: Based on daily clinical observations there was apathy, anorexia and vomiting noticed in the groups with the 3 highest doses. In the highest dose group 1 animal died on day 5. Adverse effects were confirmed both by the blood parameters and histopathological findings. In fact, the kidneys of the animals of the highest dose showed tubular necrosis and congestion together with focal presence of limited mixed population of lymphocytes and neutrophils. Additionally, there were several tubuli containing dense hyaline casts. The livers of the same animals showed congestion with diffuse degenerative changes, advanced cellular atypia and single cell necrosis of the hepatocytes.

Conclusion: **HPMPDAP has previously been shown to exert potent protective activity against various DNA virus infections in mouse models and was also demonstrated to be very well tolerated in mice. A close analogue of HMPDAP, i.e. HPMPC or Cidofovir is successfully used in humans for the treatment of life-threatening infections with the cytomegalovirus. However, much to our surprise, HPMPDAP was not well tolerated in pigs. Thus before evaluating the efficacy of novel (antiviral) compounds in pigs, the optimal treatment schemes (dose and frequency of administration) and administration routes (oral administration versus intramuscular) should be established. Additionally, our study confirms the statement that toxicological and pharmacokinetic data obtained from studies in laboratory animals cannot per se be extrapolated to the target species.**

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NOTES

NOTES

NOTES

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