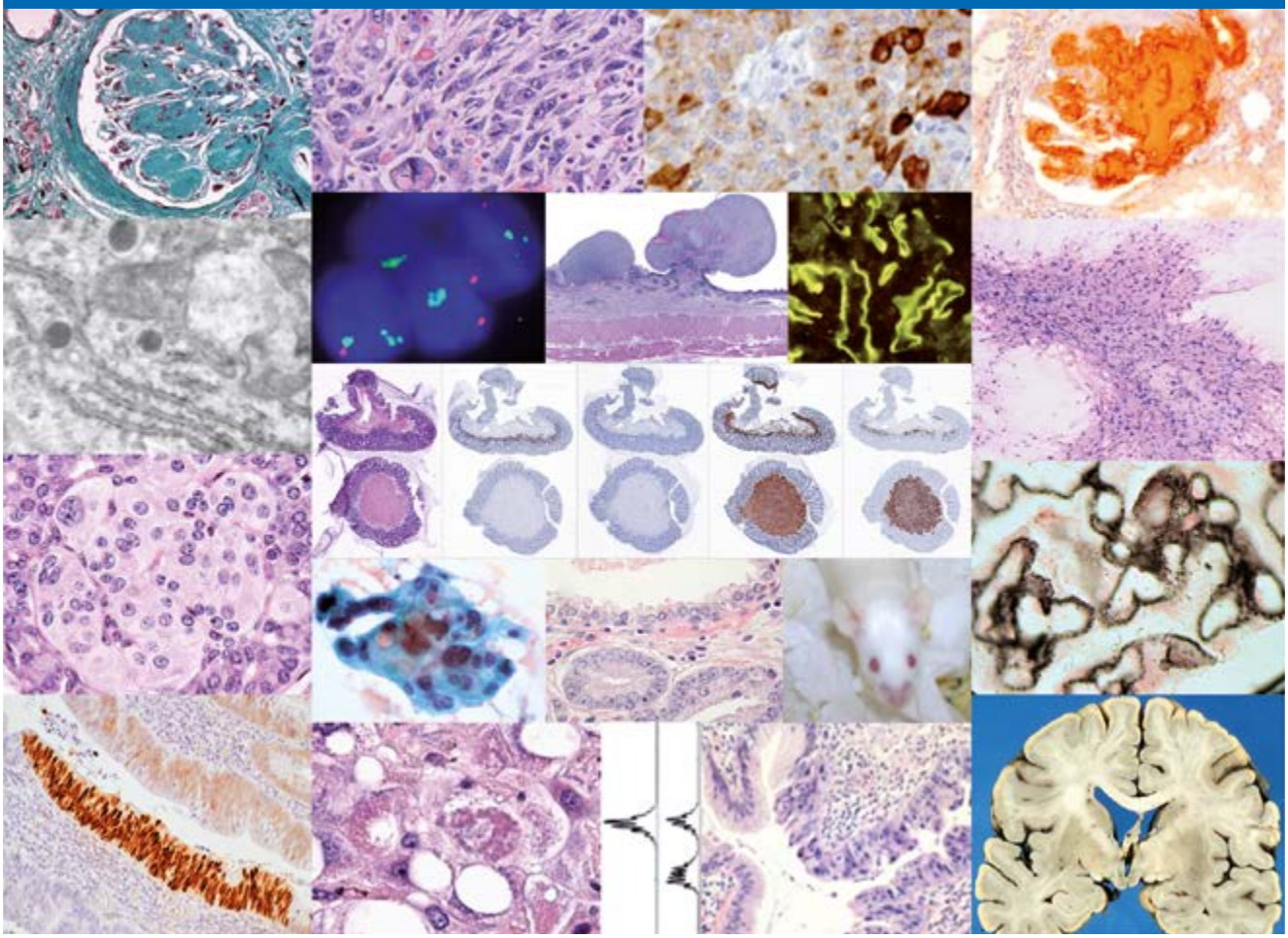
BWPath

BELGIAN WEEK OF PATHOLOGY

6th BELGIAN WEEK OF PATHOLOGY

OCTOBER 21-24, 2015

Congrescentrum Augustijnenklooster - Ghent



WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

www.belgian-society-pathology.eu

info@fresco-consult.com

www.fresco-consult.com

WELCOME

Dear Colleagues,

It is with pleasure that we welcome you at the 6th Belgian Week of Pathology. After the success of the previous meetings, we decided to organise another 4-day meeting, and once more we are hosted at Thagaste – Trefpunt Augustijnen, a magnificent monastery where the Fathers Augustinians are still living and working.

Although no other device has captured pathologists' hearts as much as the simple light microscope, the face of pathology is now changing. Technology advances rapidly, but to part of the pathologists, especially those educated in traditional methods, molecular biology can seem like a black hole. Therefore, we start this meeting with a course on molecular pathology that will be helpful for trainees as well as for established colleagues.

This year's keynote lecturers are two excellent speakers who will discuss the role pathologists can play in the diagnosis of infectious diseases, and the spectrum of neuroendocrine tumours of the lung in view of the new WHO classification. Together with the other sessions, we provide a high-level program that appeal to all clinical pathologists.

An award of 2.000€ is offered by the Boël Foundation for the best oral presentation given by a young researcher in the field of oncology. The Belgian Society of Pathology awards the best poster with a prize of 500€. Winners are announced on Friday at 16:45.

I take the opportunity to thank our partners from the industry for their renewed support; without them, this Week would never be possible...

Please enjoy the meeting as well as the monastery and the city – they might offer added value to our meeting.

Sincerely yours,

Pieter Demetter
President 6th Belgian Week of Pathology

The monastery of Saint-Stephen in Ghent

The monastery of Saint-Stephen was founded in 1296 by the Augustinian monks (Blackfriars). There was considerable financial support from the Borluut family which is depicted in Van Eyck's world famous painting "Adoration of the Mystic Lamb".

As so many other European monasteries, this Augustinian monastery had an agitated history. Iconoclasms, the rise of the protestant religion, the French revolution, the industrial revolution, wars … were all historical events seriously affecting the life of the Augustinian monks in Gent.

In 1838 the monastery was partly transformed into a textile factory, a fate of many historical buildings during the industrial revolution. During winter a large fire destroyed a part of the monastery. But Augustinians never give up. Even during the most difficult times they stayed in the monastery: it's very unique that there always have been monks in the monastery since the very beginning in 1296 until today.

Immediately after the fire, the monastery was rebuilt in Renaissance – Baroque style with full respect for the original ornaments.

This building as we still know it today has an easy floor plan: a beautiful courtyard surrounded by 4 big corridors or cloisters, giving access to the historical rooms such as the large refectory, guestrooms, calefacts (warm rooms once used for sick or old people) and meeting rooms. On the first and second floor there are many sleeping cells for the fathers and students of the monastery. Last but not least there is the beautiful church, a daily place of meditation for hundreds of visitors.

Walk through the corridors and discover a lot of artwork and large paintings. Just one example: the "The circumcision of Christ" is an important painting of the 17th century which is currently being restored.

Last but not least, the monastery has one of Europe's oldest libraries, a place recently discovered by filmmakers all over the world. The library has been saved from the big fire thanks to the thickness of the brick walls although traces of this tragic event still are visible today.

Many other monasteries in Europe are closing and get other functions. The situation of the Saint-Stephen's monastery is quite different. There are still 9 monks living in the monastery and there is an international program to attract candidate Augustinian monks. More than 20 students from different continents are currently preparing to become a monk in this monastery. A clear sign that the history of the monastery continues.

To open its doors to the outside world, the ground floor of the monastery has been transformed into a congress centre. Today it has become a meeting point for international events organised by universities, companies and the government.

Feel welcome!

INDEX




GENERAL INFORMATION

Accreditation

Accreditation has been requested for ethics and economy.
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 submit abstracts until August 30, 2015.
All abstracts were accepted as:

- Oral presentations will be presented during the Free paper Session on Wednesday from 13:00 to 14:00.
- E-Poster presentations will take place during the morning and afternoon coffee breaks of the Wednesday 21 October.

Access to the E-Posters will be possible from Wednesday to Saturday on the assigned screens in the Exhibition Area.

The Boël Foundation will award the Best Oral Presentation with a prize of 2.000€

The BWP will award the Best Poster with a prize of 500€.

Venue

Congrescentrum Augustijnenklooster
Academiestraat 1 - 9000 Gent
Conference rooms, the exhibition, poster area and registration are on the groundfloor.

Parking

Several parkings are located close the congress venue:

- Parking Ramen - Ramen - 9000 Gent
- Parking Vrijdagmarkt - Vrijdagmarkt - 9000 Gent
- Parking Poel - Sint Michielsplein - 9000 Gent

Hotels

Ghent River Hotel: Waaistraat, 5 – 9000 Ghent - Tel: +32 (0)9 266.10.10 / Fax: +32 (0)9 266.10.15
Gravensteen Hotel: Jan Breydelstraat, 35 – 9000 Ghent - Tel: +32 (0)9 225 11 50 / Fax: +32 (0)9 225 18 50

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

Belgian Society of Pathology ASBL/VZW, composed of:

- Belgian Society of Clinical Cytology (BVKC – SBCC)
- Belgian Society of Pathology (BVPA – SBAP)
- BelgianClub of Digestive Pathology

And joined by the

- 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 :	Demetter P.
Past President :	Bogers J.P.
Treasurer :	Hoorens A.
Executive Secretary :	D'Haene N.
Local Organiser :	Cuvelier C.
Councillors :	F. Dome
	A. Jouret-Mourin
	E. Sciot
	B. Weynand
	F. Willocx

HOLOGIC
The Science of Sure

One vial. Multiple options.

Your benefits from the ThinPrep® vial and Hologic's complete solution:

- **Improve laboratory efficiency**
  - Reduce hands-on and turn-around time to better leverage your human resources
  - Perform all tests from a single sample
  - Increase productivity within given space
- **Increase safety**
  - Ensure full chain of custody
  - Trust in proven clinical performance
  - Perform all tests from a single sample
  - Minimize potential for human error

Please contact us to learn more about our complete solution for Cervical Health Screening:

Diagnostic Solutions | Hologic.com | benelux@hologic.com

FOREIGN FACULTY

Bellocq J.-P.	Strasbourg, France
Berney D.	London, U.K.
Coindre J.M.	Bordeaux, France
Couvelard A.	Paris, France
Devouassoux M.	Lyon, France
Fachetti F.	Brescia, Italy
Hofman P.	Nice, France
Klijanienko J.	Paris, France
Kluin P.	Groningen, The Netherlands
La Rosa S.	Varese, Italy
Lloveras B.	Barcelona, Spain
Morreau H.	Leiden, The Netherlands
Perren A.	Bern, Switzerland
Shepherd N.	Gloucestershire, U.K.
Travis W.	New York, USA
van der Waal I.	Amsterdam, The Netherlands
Varma M.	Cardiff, U.K.

BELGIAN FACULTY

Benoy I.	Antwerp
Bogers J. P.	Antwerp
Borbath I.	Brussels
Bourgain C.	Bonheiden
Bruyneel J.	Ghent
Callens S.	Leuven
Camboni A.	Brussels
Cockelaere K.	Ieper
Colpaert C.	Antwerp
Cosyns J.P.	Brussels
Creytens D.	Ghent
Cuvelier C.	Ghent
D'Haene N.	Brussels
Dargent J.L.	Gosselies
Demetter P.	Brussels
Delvenne P.	Liège
De Paepe P.	Brugge
Dequeker E.	Leuven
de Saint Aubin N.	Brussels
De Schepper S.	Ghent
Dome F.	Liège
Dymarkowski S.	Leuven
Franke S.	Liège
Galant C.	Brussels
Haspeslagh M.	Ghent
Hauben E.	Leuven
Hoorens A.	Ghent
Jouret-Mourin A.	Brussels
Komuta M.	Brussels
Lammens M.	Antwerp
Marbaix E.	Brussels
Nagy N.	Charleroi
Noël J.-C.	Brussels
Pauwels P.	Antwerp
Praet M.	Ghent
Remmelink M.	Brussels
Salmon I.	Brussels
Sciot R.	Leuven
Stärkel P.	Brussels
Stordeur S.	Brussels
Théate I.	Gosselies
Theunis A.	Brussels
Van Criekinge W.	Ghent
van den Broeck D.	Ghent
Van Dorpe J.	Ghent
Vanwalleghem L.	Brugge
Weynand B.	UZLeuven
Willocx F.	Brussels

PROGRAM OVERVIEW

		KLOOSTERGANGEN ROOM Ground floor	AUGUSTIN ROOM Ground floor – 200 pax	HIPPO/CARTAGHUE Ground floor – 120 pax
WEDNESDAY October 21	8:30 - 8:35	Exhibition Area Posters		Opening : P. Demetter
	8:35 - 10:00	Exhibition Area Posters		Postgraduate Course
	10:00 - 10:30	Coffee Break **e-Posters Session**		
	10:30 - 12:00	Exhibition Area Posters		Postgraduate Course
	12:00 - 13:00	**LUNCH** Exhibition Area Posters		**12:15 – 12:45** **Satellite Symposium** **MULTIPLICOM**
	13:00 - 14:00	Exhibition Area Posters		Free paper Session
	14:00 - 15:30	Exhibition Area Posters		Postgraduate Course
	15:30 - 16:00	Coffee Break **e-Posters Session**		
	16:00 - 17:15	Exhibition Area Posters		Postgraduate Course
THURSDAY October 22	8:15 - 9:00		**8:15 - 9:00** **Breakfast Satellite Symposium LEICA**	
	9:00 - 10:00	Exhibition Area Posters	Dermatopathology: Cutaneous lymphoproliferative diseases	8:30 - 10:00 Ethics and Economy: Second lecture in oncology
	10:00 - 10:30	Coffee Break Exhibition Area / Posters		
	10:30 - 12:00	Exhibition Area/Posters	Dermatopathology: Cutaneous lymphoproliferative diseases	Ethics and Economy: Second lecture in oncology
	12:00 - 12:45	Exhibition Area/Posters	Keynote Lecture In memoriam E. Van Marck P. Hofman (Nice, France)	
	12:45 - 14:00	**LUNCH** Exhibition Area Posters	**13:15 - 14:00** **Satellite Symposium** **ROCHE diagnostics**	
	14:00- 15:30	Exhibition Area/Posters	Head & Neck Pathology	Non-neoplastic Hepatopathology
	15:30 - 16:00	Coffee Break Exhibition Area		
	16:00 - 17:30	Exhibition Area/Posters	Head & Neck Pathology	Non-neoplastic Hepatopathology
	17:30 - 18:30	**Drink, Cheese & Wine**		

PROGRAM OVERVIEW

		KLOOSTERGANGEN ROOM Ground floor	AUGUSTIN ROOM Ground floor – 200 pax	HIPPO/CARTAGHUE Ground floor – 120 pax
FRIADAY October 23	9:00 - 10:30	Exhibition Area Posters	Neuroendocrine tumours in daily practice	Symposium Flemish Collaborative Glomerulonephritis Group
	10:30 - 11:00	Coffee Break Exhibition Area / Posters		
	11:00 - 12:00	Exhibition Area Posters	Neuroendocrine tumours in daily practice	
	12:00 - 13:00	Exhibition Area Posters	Keynote Lecture W. Travis (New York, USA)	
	13:00 - 14:00	**LUNCH** Exhibition Area Posters	**13:15 – 14:00 Satellite Symposium ASTRAZENECA diagnostics**	
	14:00 - 15:30	Exhibition Area Posters	Ovarian sex cord/stromal tumours	
	15:30 - 16:00	Coffee Break Exhibition Area / Posters		
	16:00 - 16:45	Exhibition Area Posters	Ovarian sex cord/stromal tumours	
	16:45 - 17:00		**BWP 2015 Awards :** Boël Prize / Best Poster	
	17:00 - 18:00			General Assembly Belgian Society of Pathology
SATURDAY October 24	9:00 - 10:00	Exhibition Area	Surgical Pathology: Selected topics	Program for Cytotechnologists
	10:00 - 10:30	Coffee Break Exhibition Area		
	10:30 - 12:00	Exhibition Area	Surgical Pathology: Selected topics	Program for Cytotechnologists
	12:00 - 13:00	**LUNCH** Exhibition Area Posters	**12:00 – 12:45 Satellite Symposium BIOCARTIS**	
	14:00 - 16:00	Exhibition Area	Soft tissue pathology	
	16:00-16:05	Exhibition Area	Closing: P. Demetter	

Multiplicom

Enabling personalized medicine

Multiplicom's MASTR™ technology: enabling next-generation pathology

Multiplicom Symposium

21 October 2015
12:15-12:45
Room Hippo

Dr. Wouter Bossuyt
Product Specialist Oncology, Multiplicom

Dr. Gaëlle Boulet
Research Manager, Multiplicom

Next-generation sequencing (NGS) is rapidly changing the way pathologist are characterizing samples in oncology. The diagnostic company, Multiplicom, is making the transition to and the implementation of NGS in pathology simple. We will introduce how Multiplicom can partner with you to implement NGS in your lab. Dr. Gaëlle Boulet will show performance data of two of our product in oncology, BRCA Tumor MASTR Plus and the Tumor Hotspot MASTR Plus. The data will include preliminary results from the CE-IVD validation study and internal verification studies.

WEDNESDAY 21 MORNING

Room Hippo

08:30-08:35: **Welcome message**
P. Demetter (Brussels)

08:35-12:00: **Postgraduate course: Molecular pathology at the service of the pathologist.**
Chairpersons: J.-P. Bogers (Antwerp), N. D'Haene (Brussels)

08:35 ***Molecular biology for dummies.***
D. van den Broeck (Ghent)

09:00 ***DNA-methylation for dummies.***
W. Van Criekinge (Ghent)

09:25 ***In situ techniques : FISH and CISH.***
J. Van Dorpe (Ghent)

Coffee break Area

10:00-10:30 ***Coffee break*** */ Morning e-Poster Presentations*

10:30 ***mRNA in situ hybridisation.***
(J.-P. Bogers (Antwerp)

11:00 ***Polymerase chain reaction techniques.***
S. Franke (Liège)

11:30 **Next generation sequencing: a new tool for molecular characterisation of Tumours.**
N. D'Haene (Brussels)

12:00-14:00 ***Lunch***

Room Hippo

12:15-12:45 Satellite Symposium: Multiplicom

WEDNESDAY 21 AFTERNOON

Room Hippo

13:00-14:00: **Free paper session**
Chairpersons: P. Demetter (Brussels), C. Bourgain (Bonheiden)

13:00 **Specimen integrity and improved margin analysis in endoscopic submucosal dissection may avoid surgery.**
A. Dessain, C. Snauwaert, H. Piessevaux, A. Jouret-Mourin / Brussels

13:10 **Tumour associated antigens in uterine cervical cancer, dysplasia and normal cervical epithelium.**
A. Eerdekens, C. Simoens, O. Schoolmeesters, J. Bogers / Antwerp

13:20 **New insights in the molecular pathogenesis of Epstein-Barr virus-positive and -negative post-transplant diffuse large B-cell lymphoma.**
J. Morscio, J. Finalet Ferreiro, D. Dierickx, G. Verhoef, I. Wlodarska, T. Tousseyn / Leuven

13:30 **Radiotherapy-induced damage to cancer-associated fibroblasts promotes colorectal cancer progression.**
J. Tommelein, L. Verset, F. Gremonprez, T. Boterberg, P. de Tullio, P. Demetter, M. Bracke, O. De Wever / Ghent, Brussels and Liège

13:40 **Significance of plasma cells in melanoma.**
F. Bosisio, N. van Baren, M. Mercier, M. Stas, J. Wouters, J. van de Oord / Leuven

13:50 **First Belgian national external quality assessment for special stains in Histopathology.**
V. Ghislain, C. Egele, H. Verbeke, W. Coucke, J. Michiels, J. Bellocq, C. Galant, P. Pauwels, M. Pétein, B. Van den Heule, C. Van Campenhout / Brussels and Strasbourg

WEDNESDAY 21 AFTERNOON

Room Hippo

14:00-17:15: **Postgraduate Course: Clinical applications of molecular testing for solid tumours.**
Chairpersons: J.-P. Bogers (Antwerp), N. D'Haene (Brussels)

14:00 **Molecular testing for colorectal cancer.**
H. Morreau (Leiden, The Netherlands)

14:30 **Molecular testing for breast cancer.**
C. Galant (Brussels)

15:00 **Molecular testing for melanoma.**
P. Pauwels (Antwerp)

Coffee break Area

15:30-16:00 ***Coffee break** / Afternoon e-Poster Presentations*

16:00 **Molecular testing for lung cancer: which test for which patient?**
P. Hofman (Nice, France)

16:45 **Importance of external quality assessment for molecular testing.**
E. Dequeker (Leuven)

The Pathology Company

Leica BIOSYSTEMS

TRANSFORMING PATHOLOGY

Together

Leica Biosystems Symposium

"The Future of Pathology"

Speaker: Chris Rhoades, UK

Advanced Staining Senior Marketing Manager

Thursday 22nd October 2015

08:15 - 09:00

Abstract:

In an ever-changing world laboratories need to achieve more with less. Increasing workloads and decreasing budgets lead to increased stress and pressure within the workplace. Leica Biosystems has a philosophy of designing product around customer feedback and needs. By designing products that can help improve efficiencies and workflow within the laboratory, Leica Biosystems moves closer to its vision and mission of 'Advancing cancer diagnostics to improve lives'. From new advances in pre-analytics, with new instrument solutions, to workflow solutions in analytics, full automation of molecular techniques, independently assessed antibody panels and advances in inventory management, to new software developments in post analytics, all overseen by a patient tracking system that identifies potential bottlenecks whilst tracking patient samples from beginning to end of the entire process. Leica Biosystems would like to introduce you to some of these new products designed to meet your needs and those of your Patients.

LeicaBiosystems.com

THURSDAY 22 MORNING

08:15-09:00: **Breakfast Session / Satellite Symposium: Leica**

Room Augustin

09:00-12:00: **Cutaneous lymphoproliferative diseases.**
Chairpersons: A. Theunis (Brussels), S. De Schepper (Ghent)

09:00 **Intravascular lymphomas and related disease.**
F. Fachetti (Brescia, Italy)

09:30 **Blastic plasmacytoid dendritic cell neoplasms.**
F. Fachetti (Brescia, Italy)

10:00-10:30 **Coffee Break**

10:30 **Slide seminar**
F. Fachetti (Brescia, Italy), J.-L. Dargent (Gosselies), I. Théate (Gosselies), P. De Paepe (Brugge), A. Camboni (Brussels), M. Haspeslagh (Ghent)

Room Hippo

08:30-12:00: **Ethics and Economy: Second lecture in oncology**
Chairpersons: I. Salmon (Brussels), A. Jouret-Mourin (Brussels)

08:30 **The KCE report on organisation of care for adults with a rare or complex cancer : context and importance for Belgian pathologists.**
S. Stordeur (Brussels) and K. Cokelaere (Ieper)

09:00 **Second lecture: the French experience.**
J.-P. Bellocq (Strasbourg, France)

09:30 **Second lecture: the Dutch experience.**
P. Kluin (Groningen, The Netherlands)

10:00-10:30 **Coffee break**

10:30 **Second opinions in genito-urinary pathology: experiences from London.**
D. Berney (London, U.K.)

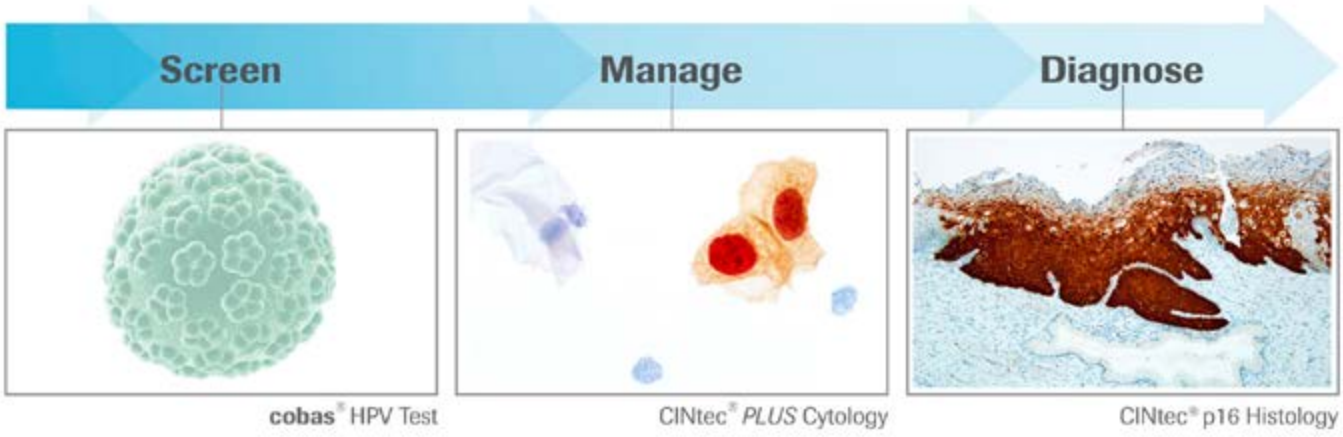
11:00 **Legal aspects of virtual pathology and of second opinions.**
S. Callens (Leuven)

11:30 **Discussion – Round table**

Room Augustin

12:00-12:45: **Keynote lecture : In Memoriam E. Van Marck**
Chairperson: M. Lammens (Antwerp)

Role of the pathologist in the diagnosis of infectious diseases.
P. Hofman (Nice, France)



Together, let's stop Cervical Cancer

HPV screening programs are being established around the world in order to improve our ability to prevent cervical cancer. The combination of **cobas**® HPV molecular tests and dual detection of p16 and Ki67 (CINtec® *PLUS* Cytology), respectively for screening and triage of women around the world, offers the greatest probability of pre-cancerous lesion detection and reduction of mortality as demonstrated in a health economic model.

Join us at the Roche Satellite Symposium to discuss how Europe and Belgium are responding to the challenges of women's screening and triage with the introduction of new, innovative, unique technologies.

Event: Roche Satellite Symposium
Location: Augustin Room
Date: Thursday, October 22nd
Time: 13:15 – 13:45

THURSDAY 22 AFTERNOON

12:45-14:00 **Lunch**

Room Augustin

13:15-14:00: **Satellite Symposium: Roche Diagnostics**

14:00-17:00: **Head and Neck Pathology**
Chairpersons: B. Weynand (Leuven), F. Dome (Liège)

14:00 **Pathology of salivary glands.**
J. Klijanienko (Paris, France)

15:00 **Head and neck carcinoma and HPV.**
B. Lloveras (Barcelona, Spain)

15:30-16:00 **Coffee break**

16:00 **Odontogenic cysts of the jaws.**
I. van der Waal (Amsterdam, The Netherlands)

16:45 **Slide seminar: Head and Neck pathology**
M. Remmelink (Brussels), M. Praet (Ghent), E. Hauben (Leuven), D. Creytens (Ghent), Jasper Bruyneel (Ghent), Lieve Vanwalleghem (Brugge).

Room Hippo

14:00-17:30: **Non-neoplastic Hepatopathology**
Chairpersons: M. Komuta (Brussels), P. Demetter (Brussels)

14:00 **Transjugular or transcutaneous liver biopsy: indications, technical aspects and expectations from the hepatologist.**
T. Sersté (Brussels)

14:45 **Origin and significance of macrovesicular and microvesicular steatosis.**
N. Nagy (Charleroi)

15:30-16:00 **Coffee break**

16:00 **CT and MRI of diffuse liver disease.**
S. Dymarkowski (Leuven)

16:45 **Clinicopathological aspects of drug-induced hepatitis.**
P. Starkel and M. Komuta (Brussels)

17:30-18:30 **Drink, Cheese & Wine**

FRIDAY 23 MORNING

Room Augustin

9:00-12:00: **Neuroendocrine tumours in daily practice**
Chairpersons: A. Hoorens (Brussels), I. Salmon (Brussels)

09:00 **Pitfalls in diagnosis of neuroendocrine tumours of the lung.**
W. Travis (New York, USA)

09:30 **Gastric ECL-cell tumours and related precursor lesions.**
S. La Rosa (Varese, Italy)

10:00 **Intestinal NET, NEC and MANEC.**
A.Perren (Bern, Switzerland)

10:30-11:00 **Coffee break**

11:00 **Neuroendocrine tumours of the pancreas: classification and prognostic stratification.**
A. Couvelard (Paris, France)

11:30 **The DNET registry, a prospective, national, online registry of digestive neuro-endocrine tumours: status after 30 months of inclusion.**
I. Borbath (Brussels)

Room Hippo

09:00-10:30 **Symposium Flemish Collaborative Glomerulonephritis Group (FCGG)**
Chairpersons: S. Rorive (Brussels), E. Lerut (Leuven)

09:30 **Presentation Flemish Collaborative Glomerulonephritis Group.**
W. Laurens (Sint-Niklaas & U Gent)

09:20 **Classification of glomerulonephritis.**
A. Dendooven (Antwerp)

09:50 **Practical implementation and examples.**
E. Lerut (Leuven)

10:30-11:00 **Coffee break**

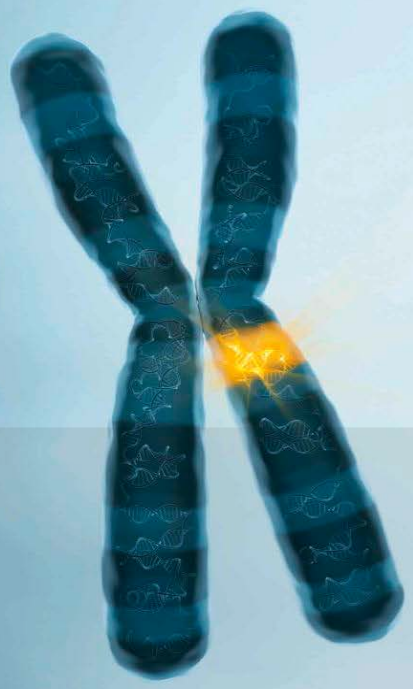
Room Augustin

12:00-13:00: **Keynote lecture**
Chairperson: B. Weynand (Leuven)

The spectrum of neuroendocrine tumours of the lung in the view of the new WHO classification.
W. Travis (New York, USA)

NOTES

Ovarian cancer in the new era of targeted therapy



Belgian Week of Pathology - AstraZeneca satellite symposium
Friday 23 October 2015, 13h15 – 14h00
Room Augustin, Augustijnenklooster Ghent

With the expected availability of Lynparza® (olaparib) therapy for patients with BRCA-mutated (germline or somatic) high-grade serous ovarian carcinoma* there is a renewed interest in the precise identification of this histologic type of ovarian carcinoma.

Olaparib: from clinical data to clinical experience
Professor Peter Vuylsteke, Oncology department, CMSE Namur

Histological classification of ovarian cancer types indicated for BRCA testing and olaparib therapy
Professor Philippe Moerman, Pathology lab, UZ Leuven

Questions and Answers

* Lynparza® (olaparib) is indicated as monotherapy for the maintenance treatment of adult patients with platinum sensitive relapsed BRCAm ovarian cancer (including fallopian tube or primary peritoneal) who are in response (complete response or partial response) to platinum-based chemotherapy.

iD.BRCA

AstraZeneca

FRIDAY 23 AFTERNOON

13:00-14:00 **Lunch**

Room Augustin

13:15-14:00 **Satellite Symposium: AstraZeneca**

Room Augustin

14:00-16:45 **Ovarian sex cord/stromal tumours : morphology, immunohistochemistry and genetic profile.**
Chairpersons: P. Delvenne (Liège), J.-C. Noël (Brussels)

14:00 **Pure sex cord tumours.**
M. Devouassoux (Lyon, France)

14:45 **Pure stromal tumours.**
J.-C. Noël (Brussels)

15:30-16:00 **Coffee break**

16:00 **Mixed sex cord-stromal tumours.**
E. Marbaix (Brussels)

Room Augustin

16:45-17:00 **BWP 2015 Awards:**
Boël Prize: Best Oral Presentation
BWP Prize: Best E-Poster Presentation

Room Hippo

17:00-18:00 **General Assembly Belgian Society of Pathology.**

SATURDAY 24 MORNING

Room Hippo

09:00-12:00: **Program for cytotechnologists**
Chairpersons: F. Willocx (Brussels), J.P. Bogers (Antwerp)

09:00 **Cytology with or without knowledge of HPV: is there a place for co-testing?**
J.-P. Bogers (Antwerp)

09:30 **HPV detection methods for cytotechnologists.**
I. Benoy (Antwerp)

10:00-10:30 **Coffee break**

10:30-12:00 **Slide seminar**

Room Augustin

09:00-12:00: **Surgical pathology : selected topics**
Chairpersons: C. Cuvelier (Ghent), N. D'Haene (Brussels)

09:00 **The molecular classification of breast cancer: implications for the surgical pathologist.**
C. Colpaert (Antwerp)

09:30 **Management of lung cancer biopsies in 2015.**
B. Weynand (Leuven)

10:00-10:30 **Coffee break**

10:30 **The pathology of bowel cancer screening.**
N. Shepherd (Gloucestershire, U.K.)

11:00 **Gleason grading : an update + quiz.**
M. Varma (Cardiff, U.K.)

12:00-12:45 **Satellite Symposium: Biocartis**

6th Belgian Week of Pathology

Belgian Society of Pathology

SATURDAY
OCTOBER 24th 2015
12:00h until 12:45h
in the Augustin Room

High precision diagnostics for high precision medicine

Invitation to **Biocartis workshop**

Program

Dr. Claire Bourgain Imelda Ziekenhuis Bonheiden	Early adoption of the novel fully automated Idylla™ system directly from FFPE samples in a peripheral laboratory setting.
Prof. Nicky D'Haene ULB	KRAS Beta-trial on the Idylla™ system and a vision on the co-existence of the Idylla™ system with next generation sequencing.
Olivier Poulin Biocartis	Monitoring of treatment response and early signs of progression and resistance by clinicians with Idylla™.

BIOCARTIS

Brochures?
Scan here

NOTES

SATURDAY 24 AFTERNOON

12:00-14:00 **Lunch**

Room Augustin

14:00-16:00: **Soft tissue pathology**
Chairpersons: N. De Saint Aubain (Brussels), R. Sciot (Leuven)

14:00 **Pathology networks and databases for soft tissue tumours.**
J.M. Coindre (Bordeaux, France)

14:40 **Slide seminar**
J.M. Coindre (Bordeaux, France), R. Sciot (Leuven), N. De Saint Aubain (Brussels), D. Creytens (Ghent)

16:00-16:05 **Closing of the BWP 2015**
P. Demetter (Brussels)

EXHIBITION FLOOR

EXHIBITIORS

Catering		BUFFETS
		DESK / REGISTRATION / E-POSTERS
	@	Accreditation
		Conference rooms
Premium Partners	**A 1**	ROCHE Diagnostics / Pharma
Major Partners	**B1**	SECTRA
	B2	SYSMEX
	B3	THERMO SCIENTIFIC
Regular Partners	**C 1**	BIOCARTIS
	C 2	MENARINI Diagnostics
	C 3	PHILIPS
	C 4	SAKURA
	C 5	LEICA
	C 6	ASTRAZENECA Diagnostics
	C 7	DAKO
	C 8	PROPATH
	C 9	VISIOPHARM
	C 10	HOLOGIC
	C 11	ABBOTT
	C 14	PROMEGA
	C 15	MULTIPLICOM
	C 16	HAMAMATSU
Booksellers	**D1**	MEDITHEQUE

Promotional offer valid until December 15, 2015

Ci-E: Upright motorized Microscope dedicated for Pathology

~~16,523,65 € eVAT~~ **9.250,00 € eVAT**

No contractual picture

✓ **Advanced Automation Features:**

- Fast motorized switching of objective position
- Fast synchronized motorized swing-out lens movement allowing change from 2x to higher objective
- The light intensity is automatically adjusted to the objective
- Remote control with buttons for your preferred microscope settings

✓ **Ergonomy:**

- Tilting Telescopic Head adapted to your body type in order to ensure that the microscope fits you perfectly rather than you being forced to fit the microscope.
- Capture button located on the microscope allowing to capture images without removing your eyes from the eyepieces.
- Manually changing of objectives is replaced by a simple touch of a button
- LED eco-illumination (with light quality as with halogen)

✓ **Superior Optical System:**

- The incredible view provided by Nikon's proprietary CFI60 optics is now enhanced with new patent pending "fly-eye" technology. With Nikon's CFI60 optics, the brightest, sharpest, most uniform crystal-clear images can be obtained even in high magnification. This offer is based on the high grade Plan Fluor series maximizing brightness and numerical aperture.

Your local contact from Nikon Belux :
Philippe Baert & Jean-Baptiste Braquenier
microscience@nikon.be

This offer is including the following items:
ECLIPSE Ci-E frame with cover , Ceramic-coated Stage C-SR2S , Tilting Telescopic Head C-TE2 , eyepieces 25 mm CFI UW 10X/25, Motorized Swing-out condenser CI-C-E , daylight filter 45 mm, CFI plan achromat objective UW 2X/0.06, plan fluor 4X, 10X, 20X, 40X, Tube for analyzer C-ISA, Polariser C-SP, camera is optional

INVITED LECTURES

INVITED LECTURES

WEDNESDAY OCTOBER 21

L 01	D. van den Broeck (Ghent)	Molecular biology for dummies.	
L 02	W. Van Criekinge (Ghent)	DNA-methylation for dummies.	
L 03	J. Van Dorpe (Ghent)	In situ techniques : FISH and CISH.	
L 04	J.-P. Bogers (Antwerp)	mRNA in situ hybridisation.	
L 05	S. Franke (Liège)	Polymerase chain reaction techniques.	34
L 06	N. D'Haene (Brussels)	Next generation sequencing: a new tool for molecular characterisation of Tumours.	38
L 07	H. Morreau (Leiden, The Netherlands)	Molecular testing for colorectal cancer.	44
L 08	C. Galant (Brussels)	Molecular testing for breast cancer.	45
L 09	P. Pauwels (Antwerp)	Molecular testing for melanoma.	51
L 10	P. Hofman (Nice, France)	Molecular testing for lung cancer: which test for which patient?	54
L 11	E. Dequeker (Leuven)	Importance of external quality assessment for molecular testing.	

THURSDAY OCTOBER 22

L 12	F. Facchetti (Brescia, Italy)	Intravascular lymphomas and related disease.	
L 13	F. Facchetti (Brescia, Italy)	Blastic plasmacytoid dendritic cell neoplasms.	
L 14	S. Stordeur (Brussels) & K. Cokelaere (Ieper)	The KCE report on organisation of care for adults with a rare or complex cancer : context and importance for Belgian pathologists.	55
L 15	J.-P. Bellocq (Strasbourg, France)	Second lecture: the French experience.	
L 16	P. Kluin (Groningen, The Netherlands)	Second lecture: the Dutch experience.	61
L 17	D. Berney (London, U.K.)	Second opinions in genito-urinary pathology: experiences from London.	
L 18	S. Callens (Leuven)	Legal aspects of virtual pathology and of second opinions.	63
L 19	P. Hofman (Nice, France)	Role of the pathologist in the diagnosis of infectious diseases.	67
L 20	J. Klijanienko (Paris, France)	Pathology of salivary glands.	68
L 21	B. Lloveras (Barcelona, Spain)	Head and neck carcinoma and HPV.	69
L 22	I. van der Waal (Amsterdam, The Netherlands)	Odontogenic cysts of the jaws.	72
L 23	T. Sersté (Brussels)	Transjugular or transcutaneous liver biopsy: indications, technical aspects and expectations from the hepatologist.	
L 24	N. Nagy (Charleroi)	Origin and significance of macrovesicular and microvesicular steatosis.	79
L 25	S. Dymarkowski (Leuven)	CT and MRI of diffuse liver disease.	93
L 26	P. Starkel & M. Komuta (Brussels)	Clinicopathological aspects of drug-induced hepatitis.	94

INVITED LECTURES

FRIDAY OCTOBER 23

L 27	W. Travis (New York, USA)	Pitfalls in diagnosis of neuroendocrine tumours of the lung.	95
L 28	S. La Rosa (Varese, Italy)	Gastric ECL-cell tumours and related precursor lesions.	97
L 29	A. Perren (Bern, Switzerland)	Intestinal NET, NEC and MANEC.	103
L 30	A. Couvelard (Paris, France)	Neuroendocrine tumours of the pancreas: classification and prognostic stratification.	
L 31	I. Borbath (Brussels)	The DNET registry, a prospective, national, online registry of digestive neuro-endocrine tumours: status after 30 months of inclusion.	104
L 43	W. Laurens, Sint-Niklaas	Presentation Flemish Collaborative Glomerulonephritis Group.	145
L 32	W. Travis (New York, USA)	The spectrum of neuroendocrine tumours of the lung in the view of the new WHO classification.	105
L 33	M. Devouassoux (Lyon, France)	Pure sex cord tumours.	
L 34	J.-C. Noël (Brussels)	ure stromal tumours.	
L 35	E. Marbaix (Brussels)	Mixed sex cord-stromal tumours.	

SATURDAY OCTOBER 24

L 36	J.-P. Bogers (Antwerp)	Cytology with or without knowledge of HPV: is there a place for co-testing?	107
L 37	I. Benoy (Antwerp)	HPV detection methods for cytotechnologists.	112
L 38	C. Colpaert (Antwerp)	The molecular classification of breast cancer: implications for the surgical pathologist.	114
L 39	B. Weynand (Leuven)	Management of lung cancer biopsies in 2015.	119
L 40	N. Shepherd (Gloucestershire, U.K.)	The pathology of bowel cancer screening.	122
L 41	M. Varma (Cardiff, U.K.)	Gleason grading : an update + quiz.	134
L 42	J.M. Coindre (Bordeaux, France)	Pathology networks and databases for soft tissue tumours.	144

Highlighted authors have authorised publication of their presentation.

INVITED LECTURES

L 05

POLYMERASE CHAIN REACTION TECHNIQUES

Sabine Franke
CHU Liège

The Polymerase chain reaction (PCR) was invented by Kary Mullis in 1983.

The PCR technique has a substantial impact on the pathology practice. The PCR technique enables the identification of molecular predictive and prognostic markers, accurate diagnostics and the finding of novel molecular targets for more specific therapeutic approach and eventual drug development.

Importance of material

Crucial for the PCR analyses is the proper material. This concerns the pre-analytical step (fixation of the material) and the adequate amount of non-necrotic tissue.

The quality of the starting material affects the quality and yield of the isolated deoxyribose nucleic acid (DNA). The highest DNA yield and quality is achieved by purifying genomic DNA from freshly harvested tissues and cells. If samples cannot be processed immediately after harvesting, they should be stored under conditions that preserve DNA integrity. In general, genomic DNA yields will decrease if samples are stored at either 2–8°C or –20°C without previous treatment. In addition, repeated freezing and thawing of frozen samples should be avoided as this will lead to genomic DNA of reduced size or to reduced yields of pathogen DNA (e.g., viral DNA).

PCR is very sensitive and can be applied on small samples. If the tumor on the biopsy is not homogenous present, eventually a macrodissection can be performed to increase the presence of the malignant cells in the sample.

Nucleic acids for the PCR

DNA can be purified using many different methods and protocols.

DNA is a relatively stable molecule. However, introduction of nucleases to DNA solutions should be avoided as these enzymes will degrade DNA. Genomic DNA consists of very large DNA molecules, which are fragile and can break easily. To ensure the integrity of genomic DNA, excessive and rough pipetting and vortexing should be avoided. DNA is subject to acid hydrolysis when stored in water, and should therefore be stored in TE buffer.

The concentration and purity of DNA should be determined in a spectrophotometer. Nucleic acids and proteins have absorbance (optical density - OD) maxima at 260 and 280 nm, respectively. Pure DNA has an OD260/OD280 ratio of ~1.8. Lower ratios could be caused by protein or phenol contamination.

Qualitative PCR

Polymerase chain reaction (PCR) is a method that allows exponential amplification of short DNA sequences. This DNA sequence has usually a length of 100 to 600 bases and contains the region of interest. During a typical PCR, DNA is mixed with deoxynucleotides (dNTPs), Magnesium, a DNA polymerase and primers. Each of the primers is about 20 nucleotides in length, which are complementary to a defined sequence on each of the two strands of the DNA. These primers are extended by the DNA polymerase (Taq polymerase) to copy the designated sequence. After making this copy, the same primers can be used again, not only to make another copy of the input DNA strand but also of the short copy made in the first round of synthesis. In theory, each PCR cycle doubles the amount of amplicon in the reaction (exponential amplification).

There are three major steps in a PCR, which are repeated in 25 to 40 cycles. This is done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time.

1. **Denaturation**: double-stranded DNA template is denatured.
   At usually 94-96°C the double strand DNA melts by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

2. **Annealing:** primers anneal to the single-stranded DNA template.
   The temperature is decreased to the annealing temperature (typically 50–72 °C). This allows the primers to anneal to the complimentary single-stranded DNA template. This temperature must be low enough to allow hybridization of the primer to the strand, but high enough for the hybridization to a perfectly complementary part of the template. If the temperature is too low, the primer could bind imperfectly. If it is too high, the primer might not bind.
   Additionally, the DNA polymerase binds the double-stranded DNA at this step and initiates DNA synthesis.

3. **Extension:** synthesis and elongation of the new target DNA strand.
   During the extension step (typically 68-72°C) the DNA polymerase extends the primer. The polymerase couples dNTPs (complementary to the template) to the primer in one direction of the DNA. This duplicates the DNA fragment between the primers. After the first few rounds of synthesis, the length of the amplified DNA is limited by where each primer binds, thus nearly all amplified DNA will be as long as the distance between the two primers on the template DNA.

The reaction is held at 70-74°C for several minutes after the last PCR cycle to allow any remaining single stranded DNA to be fully extended. The reaction is complete and the resulting amplified fragments are held at a low temperature (~4°C) until analysis.

Since the reaction is essentially exponential and since each cycle takes only a few minutes, a large quantity of DNA can be produced for analysis in a few hours.

The amplified product after 25-40 cycles may be analyzed by many different methods. Often the PCR product is analyzed by gel electrophoresis. The amplified DNA is run into a gel and stained with ethidium bromide. Ethidium bromide is a dye that binds to double stranded DNA by intercalation between the base pairs. The PCR product can be visualized under UV light.

A lot of analyses of interest can be performed by PCR. Important is for each PCR analysis to use controls. Controls help to detect contamination, pipetting errors, technical problems, and false negative results. For these reasons, in each PCR a 'no-template' control and a positive control should be included.

Quantitative PCR

Real-time quantitative PCR (RT-PCR) is extremely accurate, highly sensitive and less labor-intensive than current qualitative PCR methods.

Reactions are run in real-time PCR instruments with thermal cycling and fluorescence detection capabilities. The technique is used to determine whether a specific sequence is absent or present and in how many copies. RT-PCR reactions use the same components as standard PCR reactions, but have in addition a fluorescent label that creates a signal during the PCR reaction. There exist two types:

1) Non-specific fluorescent dyes that intercalate with any double-stranded DNA.
2) Sequence-specific DNA probes consisting of oligonucleotides that are labeled with a fluorescent reporter which permits detection only when the probe is hybridized with its complementary sequence.

RT-PCR offers the ability to monitor the real-time progress of the PCR product via fluorescent detection. Fluorescence is measured during each cycle; the amount of fluorescence is proportional to the amount of PCR product.

There are two types of quantification of the PCR products: one is absolute quantification which requires a standard curve with serial dilutions of a known template. Here the PCR product is quantified based on the known quantity of the dilutions. The other one is relative quantification which determines the quantify differences between different samples.

Real-time PCR offers a rapid analysis (post-PCR step eliminated), accuracy and a high degree of sensitivity and is suited for high throughput. The possibilities are broad and for a lot of application are commercial kits available.

Reference:

- Ma et al. Application of Real-time Polymerase Chain Reaction (RT-PCR). The Journal of American Science 2006; 2(3): 1-15.
- www.uptodate.com
- www.lifetechnologies.com
- www.medicinenet.com
- www.qiagen.com
- www.wikipedia.org
- www.lifetechnologies.com

INVITED LECTURES

L 06

NEXT GENERATION SEQUENCING: A NEW TOOL FOR MOLECULAR CHARACTERISATION OF TUMOURS

N. D'Haene, Brussels

International efforts to quantify and catalogue mutations, gene expression and epigenetic data for multiple forms of cancer, coupled with the successes of targeted agents in patients with molecularly defined tumors and improvements in genomic technology, have increased enthusiasm to adopt genomic profiling into clinical cancer practice. Studies of genomic or transcriptomic profiles have led to the discovery of new biomarkers, some of them now already used in clinical daily practice. The development of tyrosine kinase inhibitor treatments has made it important to test cancer patients for clinically significant gene mutations that influence the benefit of treatment. Identification of cancer-associated mutations has become standard care for cancer treatment; examples of such include RAS mutations in metastatic colorectal carcinomas or EGFR mutations in lung cancer. Routine EGFR somatic mutation testing is now recommended in Europe and United States for non-squamous non small cell lung carcinoma (NSCLC) [1, 2]. New European guidelines strongly encourage a wide coverage of exons 18-21. Moreover, new NCCN Guidelines for NSCLC strongly endorses broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials (NCCN guidelines http://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf). Until recently, indications for standard-of-care molecular testing in colorectal carcinomas included testing for KRAS mutational status as a predictor of response to anti–EGFR agents such as cetuximab. Now, guidelines recommend that at the very least, exon 2 KRAS mutation status should be determined and whenever possible, non-exon 2 KRAS and NRAS mutation statuts should be also detemined (NCCN guidelines http://www.nccn.org/professionals/physician_gls/pdf/colon.pdf). This underlines that the number (or the extent) of biomarkers that will be need to be assessed in clinical daily practice in molecular pathology is rapidly increasing. This calls for the implementation of methods that probe the mutational status of multiple genes. Moreover, this increase in the number of genes to test is associated with a decrease in the sample size. The pathologist is facing a new challenge: optimization of available tumor tissue. As the numbers of clinically significant genetic variants have increased, clinical testing has evolved, moving from single mutations to multiplex hotspot evaluations in multiple cancer genes. The requirements for clinical sequencing include that (i) the test must be performed on routine samples with low DNA content, (ii) the test results must be delivered rapidly, (iii) the test results must be accurate and facilitate clinical decision making. Recently, next generation sequencing (NGS) has begun to supplant other technologies for gene panel sequencing. This technology enables the simultaneous sequencing of millions of short DNA sequences and offers benefits relating to lower costs and enhanced sensitivity in mutation detection. Moreover, NGS can be performed using DNA from FFPE tissue blocks.

Sequencing "throughput" refers to the amount of DNA which can be read with each sequencing reaction. Sanger sequencing, developed in the 1970s and the most widely used method of DNA sequencing, has a low throughput. In contrast, NGS enables the simultaneous sequencing of millions of short DNA sequences and therefore dramatically increases the sequencing throughput. Applications of NGS in oncology include whole genome sequencing, whole exome sequencing, targeted sequencing or RNA sequencing.

Different NGS platforms, based on different sequencing technologies, are now commercially available. Even if the precise sequencing process varies depending on the NGS platform used, the workflow is similar [3-5] :

1. Creation of a sequencing library

There are two main methods of creating a DNA library: a targeted amplicon-based approach or a hybridisation-capture approach. Targeted amplicon-based sequencing is based on multiplex polymerase chain reaction (PCR) with primers covering hotspot regions of genes of interest. Hybridisation-capture enables the analysis of larger amounts of DNA without the limitation of knowing the precise sequence of the flanking regions.

2. Preparation of the templates

The next step is to amplify the DNA so that the signal is strong enough to be detected during the sequencing. This is done by PCR and is called clonal amplification. The exact details of the PCR technique used vary on the sequencing platform, e.g. Roche and Life Technologies use emulsion-PCR and Illumina uses bridge-PCR.

3. Sequencing

The next step consists in the sequencing of the DNA templates by adding nucleotides sequentially. When a nucleotide is incorporated, a signal is produced. The different sequencing platforms use different techniques to detect this signal, based on light emission, fluorescence or variation in pH for the Roche 454, Illumina or Ion Torrent platforms, respectively.

4. Base-calling

This step converts the raw data produced by the sequencing instrument into sequences of bases. This step creates file containing millions of reads, e.g. short sequences of nucleotides.

5. Reference mapping

This step involves the alignment of the reads on a reference genome sequence. This is used to perform the variant calling analysis and to visualise the sequence reads in genome browsers such as the Integrative genomics viewer (IGV).

6. Variant calling

The variant calling step consists in the identification of the variations in the sequenced DNA in comparison to the reference genome sequences. One of the challenges is identifying true variants and separating them from sequencing noise.

7. Data interpretation

One other challenge using NGS is to interpret the detected mutations within the biological context. The variants can be grouped in three categories [6]: (i) those that may have a direct impact on patient care and are considered actionable; (ii) those that may have biological relevance but are not clearly actionable; and (iii) those that are of unknown significance. The clinical application of NGS in cancer is the detection of clinically actionable genetic/genomic alterations that are critical for cancer care. These alterations can be of diagnostic, prognostic, or therapeutic significance. There are several databases that can be accessed to evaluate the clinical significance of mutations. The most common databases used are the Catalog of Somatic Mutations in Man (COSMIC) (http://cancer.sanger.ac.uk/cosmic) and The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov/). Moreover, different databases such as MD Anderson's Personalized Cancer Therapy (https://pct.mdanderson.org), Vanderbilt's My Cancer Genome (www.mycancergenome.org) give prognostic and theranostic information.

A major advantage of NGS over traditional mutation detection methods is the ability to screen multiple mutations in multiple genes simultaneously without the need to perform several sequential tests. Several studies have already validated the use of NGS and its superiority in term of sensitivity, speed and cost compared to traditional methods [7, 9-18]. In our own experience [7, 8], for tests including more than two to three different hotspots, NGS is cheaper, faster and requires less DNA than would be needed for traditional methods. This is of particular importance for cytology samples and small-tissue biopsies for which several molecular alterations need to be screened, as is the case for NSCLC samples.

Many studies have already validated the use of NGS. Several of these studies have compared results obtained on lung cancer specimens with NGS performed on different platforms (Ion Torrent, Illumina or Roche 454 GS junior) with conventional methods including Sanger sequencing, pyrosequencing or quantitative PCR [7, 9-18]. All studies reported a 96 to 100% concordance between NGS and conventional testing for the detection of clinically relevant mutations and conclude that NGS is robust and reliable. Several of these studies mentioned that NGS outperforms Sanger sequencing in terms of sensitivity and/or time and cost. Most of the studies validated NGS on FFPE samples. De Biase et al. have focused on small routine samples including small biopsies and cytology and they have shown that NGS performed better than Sanger sequencing for the detection of mutation in the EGFR gene particularly for sample with low tumor cell content.

In addition several studies have validated the use of NGS on Fine Needle aspiration (FNA) specimens on Illumina or on Ion Torrent platform with a success rate varying from 95% to 100%.

Not only, single-nucleotide variants, small insertions and small deletions can be detected by NGS. Structural variants and copy number variants are also now possible to identify by NGS [19, 20]. Fusion genes are caused by chromosomal aberrations, e.g. inversion, translocation, large deletion or insertion. These chromosomal aberrations or copy number variants are traditionally detected through fluorescence in situ hybridization (FISH) or reverse transcriptase (RT) - PCR techniques. With NGS, fusion genes can be detected using transcriptome sequencing (RNA-Seq). Targeted c-DNA sequencing, similar to that used for genomic DNA sequencing, has recently been shown to improve the sensitivity to detect sequence variants and fusion products from RNA-seq data. Panels to investigate gene fusion detection in addition to point mutations in lung cancer were successfully designed and tested using a targeted Ampliseq RNA-sequencing approach [20].

In conclusion, NGS is intertwined with the realization of precision medicine in oncology. While this new technology does not replace the pathologic diagnosis, it allows a molecular characterization of tumours.

References

1. Kerr KM, Bubendorf L, Edelman MJ, Marchetti A, Mok T, Novello S, et al. Second ESMO consensus conference on lung cancer: pathology and molecular biomarkers for non-small-cell lung cancer. Ann Oncol. 2014 Apr 8.
2. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. Archives of pathology & laboratory medicine. 2014 Jun;137(6):828-60.
3. Gagan J, Van Allen EM. Next-generation sequencing to guide cancer therapy. Genome medicine. 2015;7(1):80.
4. Moorcraft SY, Gonzalez D, Walker BA. Understanding next generation sequencing in oncology: A guide for oncologists. Critical Reviews in Oncology/Hematology. 2015.
5. Reuter JA, Spacek DV, Snyder MP. High-throughput sequencing technologies. Mol Cell. 2015 May 21;58(4):586-97.
6. Dienstmann R, Dong F, Borger D, Dias-Santagata D, Ellisen LW, Le LP, et al. Standardized decision support in next generation sequencing reports of somatic cancer variants. Molecular oncology. 2014 Apr 4.

INVITED LECTURES

7. D'Haene N, Le Mercier M, De Neve N, Blanchard O, Delaunoy M, El Housni H, et al. Clinical validation of targeted Next Generation Sequencing for colon and lung cancers. accepted in PlosOne.
8. Le Mercier M, D'Haene N, De Neve N, Blanchard O, Degand C, Rorive S, et al. Next-generation sequencing improves the diagnosis of thyroid FNA specimens with indeterminate cytology. Histopathology. 2015 May 17;66(2):215-24.
9. Cottrell CE, Al-Kateb H, Bredemeyer AJ, Duncavage EJ, Spencer DH, Abel HJ, et al. Validation of a next-generation sequencing assay for clinical molecular oncology. J Mol Diagn. 2014 Jan;16(1):89-105.
10. Hadd AG, Houghton J, Choudhary A, Sah S, Chen L, Marko AC, et al. Targeted, high-depth, next-generation sequencing of cancer genes in formalin-fixed, paraffin-embedded and fine-needle aspiration tumor specimens. J Mol Diagn. 2013 Mar;15(2):234-47.
11. Singh RR, Patel KP, Routbort MJ, Reddy NG, Barkoh BA, Handal B, et al. Clinical validation of a next-generation sequencing screen for mutational hotspots in 46 cancer-related genes. J Mol Diagn. 2013 Sep;15(5):607-22.
12. de Biase D, Visani M, Malapelle U, Simonato F, Cesari V, Bellevicine C, et al. Next-generation sequencing of lung cancer EGFR exons 18-21 allows effective molecular diagnosis of small routine samples (cytology and biopsy). PLoS One.8(12):e83607.
13. Tops B, Normanno N, Kurth H, Amato E, Mafficini A, Rieber N, et al. Development of a semi-conductor sequencing-based panel for genotyping of colon and lung cancer by the Onconetwork consortium. BMC cancer. 2015 Jan 31;15(1):26.
14. Endris V, Penzel R, Warth A, Muckenhuber A, Schirmacher P, Stenzinger A, et al. Molecular diagnostic profiling of lung cancer specimens with a semiconductor-based massive parallel sequencing approach: feasibility, costs, and performance compared with conventional sequencing. J Mol Diagn. 2013 Nov;15(6):765-75.
15. Deeb KK, Hohman CM, Risch NF, Metzger DJ, Starostik P. Routine Clinical Mutation Profiling of Non-Small Cell Lung Cancer Using Next-Generation Sequencing. Archives of pathology & laboratory medicine. 2015 Jul;139(7):913-21.
16. Ma Y, Hendershot LM. The role of the unfolded protein response in tumour development: friend or foe? Nat Rev Cancer. 2004 Dec;4(12):966-77.
17. McCourt CM, McArt DG, Mills K, Catherwood MA, Maxwell P, Waugh DJ, et al. Validation of next generation sequencing technologies in comparison to current diagnostic gold standards for BRAF, EGFR and KRAS mutational analysis. PLoS One. 2013;8(7):e69604.

18. Tuononen K, Maki-Nevala S, Sarhadi VK, Wirtanen A, Ronty M, Salmenkivi K, et al. Comparison of targeted next-generation sequencing (NGS) and real-time PCR in the detection of EGFR, KRAS, and BRAF mutations on formalin-fixed, paraffin-embedded tumor material of non-small cell lung carcinoma-superiority of NGS. Genes, chromosomes & cancer. 2013 May;52(5):503-11.

19. Hoogstraat M, Hinrichs JW, Besselink NJ, Radersma-van Loon JH, de Voijs CM, Peeters T, et al. Simultaneous detection of clinically relevant mutations and amplifications for routine cancer pathology. J Mol Diagn. 2015 Jan;17(1):10-8.

20. Pekar-Zlotin M, Hirsch FR, Soussan-Gutman L, Ilouze M, Dvir A, Boyle T, et al. Fluorescence In Situ Hybridization, Immunohistochemistry, and Next-Generation Sequencing for Detection of EML4-ALK Rearrangement in Lung Cancer. Oncologist. 2015 Mar;20(3):316-22.

INVITED LECTURES

L 07

MOLECULAR TESTING FOR COLORECTAL CANCER

H Morreau, Department of Pathology Leiden University Medical Center

Molecular testing for colorectal cancer can be seen in the context of primary diagnosis, prognosis, compendium diagnostics and elucidation of underlying genetic causes. Illustrated by selected cases I will try to discuss the different subtopics. Although the primary diagnosis of CRC mostly does not need additional testing occasionally the disease presents itself only as a (bilateral) ovarian mass for which the differential diagnosis of primary endometrioid or mucinous ovarian carcinoma is being discussed. Prognosis profiling can lead to adjuvant treatment in stage II CRC in case of a poor prognostic profile. Compendium diagnostics testing RAS/RAF stratifies metastasized CRC for adjuvant EGFR targeting therapies. However with Next Gen Sequencing much more information is now generated that might lead to altered treatment decisions within so called "Tumor Boards". Molecular pathology can help to elucidate DNA mismatch (MMR) or base or double strand repair deficient CRC either in a sporadic or hereditary context. For suspect Lynch syndrome cancers without MLH1 hypermethylation however in > 40% of cases no germ line MMR are found, necessitating the analysis of somatic DNA variants. A similar issue is seen in APC mosaicism explaining unexplained colorectal polyposis.

L 08

Molecular testing for breast cancer

C. Galant
Cliniques Universitaires Saint Luc, Brussels

INTRODUCTION

Accurate determination of human epidermal growth factor receptor 2 (HER2) status is a critical analysis for optimizing breast cancer handling. HER2 is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family including HER1/HER3 and HER4, and controls cell proliferation. The HER2 gene is amplified in approximately 15–20% of breast cancers and gene amplification is closely linked to overexpression of the HER2 protein. Tumors with normal HER2 levels are referred to as either “HER2-normal” or “HER2-negative” (no HER2 protein overexpression/amplification), while tumors with gene amplification/overexpression are referred to as “HER2-positive”.

This presentation will be divided in two parts. A first part concerns the detection of HER2 by immunostaining (IHC) and in situ hybridization (ISH) of practical use which indicate what is positive and negative for HER2 in breast in Belgium. The second part will focused on new data providing by new molecular techniques, the recent massive parallel sequencing and other techniques focused on epigenetics, microRNA and protein expressions.

PART I: HER2 DETECTIONS

A. IHC

The IHC test gives a score of 0 to 3+ that measures the amount of HER2 receptor protein on the surface of cells in a breast cancer tissue sample. If the score is 0 to 1+, it is called “HER2 negative.” If the score is 2+, it is called "borderline." A score of 3+ is called “HER2 positive.”
However, new criteria for these IHC score determination have been defined, with changes in staining intensity as well as in percentage of positive cells.
For a positive 3+ score, the cut-off of 10% percent positive cells is reintroduced, referring to the FDA criteria for the initial clinical trials. The negative categories of score 1+ and 0 have been redefined as well, with less stringent criteria for score 0.
The equivocal category of 2+ score is broadened by including incomplete membrane staining and all other cases not defined as 0, 1 or 3+ are classified as 2+.

B. ISH

In case of an equivocal HER2 immunotesting, the confirmation of HER2 status amplification is required by an alternative method, ISH testing. Unlike other countries, an IHC 3+ score is insufficient for reimbursement of targeted therapy and then needs an ISH confirmation testing as well. ISH-positive result is defined as a HER2/CEP17 ratio >or = 2.0. Fluorescent in situ (FISHs) and more recently chromogenic in situ hybridization (CISH) or silver in situ hybridization tests

(SISH) are used with the same levels and criterias of evaluation. Only tumours characterized by an overexpression of HER2 gene are considered for trastuzumab treatment.

By the dual ISH assay methodology a result based on the HER2/CEP17 ratio is given, but an "absolute" HER2 copy number is also obtained. New guidelines recommend to opt for an unequivocal result when encountering a case of discrepant results when using single versus dual ISH algorithm. The amplification of the CEP17 region rather than a true polysomy of CEP17 has less significant issue since the absence of treatment efficacy.
In rare case of a HER2/CEP17 ratio between 1.8 and 2.2, it is recommended to count 20 extra nuclei or to retest another tumor bloc to determine an unequivocal option if possible.

However, a lot of difficulties are present for the use of these new criteria. For IHC, 2 + scoring includes now cases with circumferential membrane staining, incomplete and/or weak in more than 10% of the invasive tumor cells. In the previous guidelines, these cases were classified as 1+ , within the category weak incomplete membrane staining in any proportion of tumor cells. The new class 2+ includes also intense staining less than 10% of the cells. For Rhaka et al., there is no evidence for such a change in the diagnostic criteria, which result in a high proportion of cases requiring in situ hybridization. Another difficulty concerns the concept of histopathological concordance. It means to repeat testing on surgical specimen in particular circumstances, mainly when the grade 3 tumor provides a negative result by IHC on the core. This has financial and practical implications in the management of ISH testing. The update panel gave additionnal comments in response to these two major objections and will be issuing a revision with modification in the interpretation criteria in an upcoming new version (Hammond 2015). It will no longer indicate that grade 3 alone suffices as a criterion for mandatory retesting. The revised language will indicate that ''If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test may [emphasis added] be ordered on the excision specimen ». The panel agrees also it will be best to go back to the original definition of the HER2 IHC 2+ equivocal category to simply reflect the commonly accepted definition of ''a weak to moderate complete membrane staining is observed in >10% of tumor cells ». The concept of breast heterogeneity need also to been reevaluated.

PART II. MOLECULAR CLASSIFICATION

By using a hierarchical clustering analysis of gene expression profiling, Perou et al. (2000) were able to identify molecularly defined classes of breast cancer (luminal, HER2-enriched, basal-like and normal-like) with distinctive features. This molecular classification has been demonstrated to have a prognostic value and also to be predictive of the clinical response to adjuvant treatment. This molecular classification is explained more in detail in the presentation of C. Colpaert. The recent massive parallel sequencing and other techniques focused on abnormalities in DNA methylation, microRNA expression and protein expression, open the field and provide opportunities to characterize more completely the molecular architecture of breast cancer. The aim of this second part is to synthetize these new data provided by these new techniques published recently with relevant items in association with the molecular classification. The separate data provided by the techniques are listed in the appendix.

Luminal/ER+ breast cancers are the most heterogeneous lesions in terms of gene mutation, copy number changes and patient outcomes. However, this group shares some similar molecular characteristics:

- A high mRNA and protein expression of the luminal expression signature (GATA3, FOXA1...),
- The largest number of significantly mutated genes.
- GATA3 and FOXA1 mutated in a mutually exclusive fashion,
- A high mutation frequency of PIK3CA but PIK3 pathway not altered in luminal A cancers (PIK3 pathway highly expressed in basal-like and HER2 mRNA subtypes).
- The presence of mutation of within the p38–JNK1 pathway (MAP3K1 and MAP2K4).
- The TP53 pathway inactivated, with a low TP53 mutation frequency in luminal A.

Gene expression analysis demonstrated that markers of functional TP53 (GADD45A and CDKN1A) were highest in luminal A cancers, indicating that the TP53 pathway remains largely intact in luminal A cancers. Luminal A cancers, which have the best prognosis, are the most likely to keep activity of the tumour suppressors genes RB1 and TP53. On the other hand, TP53 is often inactivated in the more aggressive luminal B cancers and combined with MDM2 amplification and loss of cell regulation.

The HER2 subgroup is characterized by DNA amplification of HER2 in 71% of the cases, elevated mRNA and protein of HER2 and of EGFR. However, only 50% of clinically HER2 tumours fall into this HER2 mRNA subtype; the others were observed predominantly in the luminal mRNA subtypes. It indicates the presence of 2 molecular subgroups of HER2: a HER2mRNA and HER2+ subgroup characterized by higher expression of a number of receptor tyrosine kinase including EGFR, HER2 itself and genes within the HER2 amplicon and a luminal mRNA HER2+ subgroup showing luminal clusters of genes including GATA3, BCL2 and estrogen receptor.

Basal-like tumours are often referred to as triple-negative breast cancers (TNBCs) because most basal-like tumours are typically negative for ER, PR and HER2.
These tumours presented a high frequency of TP53 mutations (80%) and in genes involved in TP53 pathway. PIK3CA was the next most commonly mutated gene followed by the loss of PTEN.

Expression features of basal-like tumours include a characteristic signature containing keratins 5, 6 and 17 and high expression of genes associated with cell proliferation. High MYC activation seems to be a basal-like characteristic.

The basal-like (and TNBC) mutation spectrum was reminiscent of the spectrum seen in serous ovarian cancers (including widespread genomic instability and common gains of 1q, 3q, 8q and 12p, and loss of 4q, 5q and 8p.) with only one gene (that is, TP53) at 10% mutation frequency.

This subgroup presents the following characteristics:

- BRCA1 inactivation;

- RB1 loss and cyclin E1 amplification;
- MYC amplification and high expression;
- High frequency of TP53 mutations.

The common findings of TP53, RB1 and BRCA1 loss, with MYC amplification, strongly suggest that these are shared driving events for basal-like and serous ovarian carcinogenesis.

Bibliography:

Hammond ME, Hayes DF, Wolff AC. Clinical Notice for American Society of Clinical Oncology-College of American Pathologists guideline recommendations on ER/PgR and HER2 testing in breast cancer. J Clin Oncol,**2011**:29(15):e458.

Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF; American Society of Clinical Oncology/College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer.Arch Pathol Lab Med. **2007**;131(1):18-43

Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF; American Society of Clinical Oncology; College of American Pathologists. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J. Clin Oncol. **2013** ; 31(31):3997-4013

Lambein K, Guiot Y, Galant C, Salgado R, Colpaert C: Update of the Belgian guidelines for HER2 testing in breast cancer, BJMO **2014**; 8(4):109-15.

Rhaka EA, Pigera M, Shaaban A, Shin S, d'Alfonso T, IA Ellis, A HS Lee. National Guidelines and Level of Evidence: Comments on Some of the New Recommendations in the American Society of Clinical Oncology and the College of American Pathologists Human Epidermal Growth Factor Receptor 2 Guidelines for Breast Cancer. Journal of Clinical Oncology, **2015**;33(11):1301-2.

Hammond ME; Hicks DG. American Society of Clinical Oncology/College of American Pathologists Human Epidermal Growth Factor Receptor 2 Testing Clinical Practice Guideline Upcoming Modifications. Proof That Clinical Practice Guidelines Are Living Documents. Arch Pathol Lab Med **2015;**139(8):970-1.

Hyman DM, Solit DB. Tumor Genetic Screening Programs: A Call to Action. Journal of Clinical Oncology **2015** ;33(25):2725-6.

Kobbolt DC and 356 collaborators. The cancer genome atlas network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490(7418):61-70**.**

Appendix

A. Whole exome sequencing

Somatic mutations were identified mainly in point mutations (missense and silent mainly). A first set of genes was detected, constituted of genes already known to be implicate in breast cancer. These genes are PIK3CA (catalytic subunit of PIK3 oncogene detected is many human cancers), PTEN (human suppressor gene with a phosphatase action on cell cycle), TP53 (tumour suppressor p53), MAP3K1 (MAP3 kinase, phosphorylating enzyme playing a role in many cellular responses), GATA3 (regulator of epithelial cell differentiation in mammary gland), CDH1 (cadherin family), AKT1 (serin/threonine kinase with a role in neural development and cancers), MLL3, (myeloid/lymphoid leukemia protein) and CDKN1B (cyclin dependent kinase p27). A second major group of mutated genes newly detected by this method are:

-TBX3, TBX4 and TBX5 (T box transcription involved in mammary development or regulation of embryonic process)
- PIK3R1 (regulatory subunit) and PIK3CA (catalytic subunit) identified in glioma and endometrial cancer,
- PTPRD, member of the tyrosin phosphatase family, detected in some lung adenocarcinoma,
- RUNX1, transcription factor that regulates the differentiation of hematopoietic cells,
- CBFB, transcription factor that regulates genes of hematopoiesis (RUNX1) and osteogenesis (RUNX2),
- PTPN22, protein tyrosine phosphatase expressed in lymphoid tissue,
- CCND3, cyclin D3, a regulatory protein of the cell cycle.

Association of mutations and molecular mRNA expression

- *Correlation with genes involved:*

The overall mutation rate in luminal A class was low; with significant recurrent mutated genes more frequent within luminal A and luminal B tumours than within basal-like tumours. The most frequently mutated genes are PIK3CA (45%), followed by MAP3K1, GATA3, TP53 and CDH1.

12% of luminal A tumours presented an inactivated mutation of MAP3K1 and MAP2K4, involved in the p38 pathway. In luminal B, a wide variety of mutated genes as detected, with almost 30% of mutated PIK3CA and TP53.

In basal-like cancers, more than 80% of mutations concerned TP53. They are also characterized by an absence of almost the main mutated genes detected in luminal A and have the highest overall mutation rate.

The HER2 subtype had a hybrid pattern with a high frequency of TP53 (72%) and PIK3CA (39%) mutations and a much lower frequency of other mutated genes.

- *Correlation with types of mutations:*

In luminal A and B, the majority of mutations were non-sense whereas for basal-like and HER2 subtype, there were mainly non-sense and frame shift mutations.

CDH1 mutations were common within the lobular histological subtype and were associated with low mRNA CDH1 expression, explaining the phenotype of these lesions.

B. MicroRNA

MicroRNA (miRNA) expression analysis gives informations on miRNA expression. 7 subtypes were identified based on the 25% most variable miRNAs. The authors described a high overlap of miRNA groups 4 and 5 with the basal-like mRNA subtype and these groups contained many TP53 mutations. PIK3CA and GATA3 showed negative associations with those miRNA groups 4 and 5. The other remaining miRNA groups (1–3, 6 and 7) were composed of a mixture of luminal A, luminal B and HER2, but there was a poor correlation with mutation status.

C. DNA methylation

DNA methylation arrays identified five distinct DNA methylation groups. Group 3 showed a hypermethylated phenotype and was significantly enriched for luminal B mRNA subtype and under-represented for PIK3CA, MAP3K1 and MAP2K4 mutations. Group 5 showed the lowest levels of DNA methylation, overlapped with the basal-like mRNA subtype, and showed a high frequency of TP53 mutations, HER2-positive (HER2) clinical status, or the HER2 mRNA subtype.

D. DNA copy number

DNA copy number was assayed by SNP arrays. The data highlighted a number of significantly mutated genes within focal amplification of regions containing PIK3CA, EGFR, FOXA1 and HER2, as well as focal deletions of other regions containing PTEN or RB1. There was a correlation between these copy number DNA changes and the classical mRNA subtype. A loss of 5q and gain of 10p was characteristic in basal-like cancers. On the other hand, a gain of 1q and/or 16q loss was detected in luminal tumours.

E. Reverse phase protein arrays (RPPA)

RPPA assay determined 7 classes based on the quantification of cancer related proteins and phospho-proteins. There was a highly concordant with the mRNA subtypes, particularly with basal-like and HER2 mRNA subtypes. In the HER2 containing RPPA-defined subgroup both overexpression of HER2 and EGFR proteins with a strong concordance with phosphorylated HER2 and EGFR (pHER2 and pEGFR) proteins was noticed.In RPPA luminal tumours subgroup, there was high protein expression of ER, PR, AR, BCL2, and GATA3. These lesions matched mostly with mRNA luminal A cancers. Luminal B presented a more heterogeneous expression of proteins.

Two potentially novel protein-defined subgroups were identified, based on proteins produced by the microenvironment (stroma of the lesion) and activated fibroblasts (such as fibronectin, caveolin 1 and collagen VI) The group named reactive I consisted primarily of a subset of luminal A tumours, but the reactive II consisted of a mixture of mRNA subtypes.

L 09

MOLECULAR TESTING FOR MELANOMA

Prof. Dr. P. Pauwels, University Hospital Antwerp (UZA)

Molecular subtypes

The impact of molecular pathology on our daily pathology practice is tremendous. What was phonotypically considered as one entity, is now split up in different subtypes, based on molecular profiling. The list is long: colorectal cancer (MSI, RAS mutated, BRAF mutated, …), non small cell lung cancer (EGFR mutated, ALK rearranged), …

Also, a genomic classification of cutaneous melanoma has been introduced very recently. Based on the pattern of the most prevalent significantly mutated genes, four subtypes can be distinguished: mutant BRAF, mutant RAS, mutant NF1 and triple wild-type. A feature of the triple WT is an enrichment of KIT mutations/amplifications in the cohort.

1. BRAF subtype

The largest genomic subtype is defined by the presence of BRAF hotspot mutations. Around 50% of cutaneous melanomas harbor such mutations.

The V600E mutation is well known; but also V600K and V600R can be detected. Also, beside V600, K601 can be detected. In clinical practice, it is mandatory to use a detection system with a high sensitivity (at least 5% sensitivity).

2. RAS subtype

RAS hotspot mutations has been described in all three members of the RAS family. Some 20% show NRAS mutations, particularly Q61 mutations. Infrequently, HRAS and KRAS mutations can be found.

3. NF1 subtype

NF1 mutations can be found in 14% of samples, more than half of these mutations are predicted to be loss-of-function mutations. NF1 (coding for neurofibromin) downregulates RAS activity by stimulating the GTP-ase (natural) activity of RAS. Dysfunction of neurofibromin can be viewed as an alternative way to activate the MAPK signaling pathway.

4. Triple wild-type subtype

This is a heterogeneous subgroup characterized by a lack of hot-spot BRAF, RAS or NF1 mutations. Clinically relevant, in this group we find also KIT mutations and amplifications. In unselected cases, KIT mutations occur infrequently (2-3%); these mutations are more prevelant in mucosal and acrolentiginous melanomas. Expression of C-KIT protein does not appear to correlate with sensitivity to C-KIT inhibitors, whereas the presence of activating C-KIT gene mutations does correlate.

Therapeutic testing in melanoma

As has been stated, testing for V600 mutations is mandatory in metastatic melanomas. If only one oncogene can be tested at a time, in non-acral/non-mucosal melanomas, BRAF should be tested first, with KIT testing only be offered if BRAF is negative. In acral and mucosal melanomas especially, mutation for C-KIT should also be looked for.

An important issue is: which material should be tested; the primary lesion or the metastatic lesion? In a retrospective literature search by Varada and Mahalingam, it was concluded that 9,8% exhibit a discordant BRAF status. 7,9% of the mutated primary tumors were WT in their metastatis, while 11,5% "switched" from WT to mutated. Also, the exact localization of the metastasis seems to be important: subcutaneous and brain metastatic lesions show more discordance than for example lymph node metastasis. NRAS mutations seem to be more concordant. There are not enough data on C-KIT mutation concordance. An open question is; how does the BRAF status change in time. For example; is the status the same if a metastasis develops half a year after excision of the primary lesion vs. when the metastasis develops after five years? An exciting new method of BRAF detection is using the liquid biopsy: BRAF mutations can be detected in the plasma via analysis of circulating tumor DNA.

Metastatic melanoma in the immunomodulatory therapy area

Drugs inhibiting CTLA-4 on T-cells (ilitimumab) or targeting the programmed cell death receptor 1 (PD1) (nivolumab, pembrolizumab) have significantly improved the overall survival of patients with metastatic melanoma. PD-L1 expression (one of the ligands of PD1) on melanoma cells is believed to be induced mainly by T-cell responses mounted against the tumor and to be an important adaptive response that allows escape from immune attack. There seems to be a correlation between the BRAF mutation status and PD-L1 expression.

Unfortunately, there is a problem with the selection of an appropriate biomarker. The more expression of PD-L1 on melanoma cells is not a guarantee for response; in particular, patients with melanomas without PD-L1 expression can show significant responses. The presence of tumor infiltrating lymphocytes (TIL) should also be taken into account. This is a rapidly evolving field of investigation.

Selected reading

1. The Cancer Genome Athos Network: "Genomic classification of cutaneous melanoma." Cell 2015; 161: 1681-1696
2. Varada S, Mahalingam M: "Mutation stability in primary and metastatic melanoma: what we know and what we don't." Histol Histopathol (2015); 30: 763-770
3. Colombino M, Capone M, Lissia A et al: "BRAF/NRAS mutation frequencies among primary tumors and metastases in patients with melanoma." J Clin Oncol (2012); 30: 2522-2529
4. Tsao S, Weiss J, Hudson C et al: "Monitoring response to therapy in melanoma by quantifying circulating tumour DNA with droplet digital PCR for BRAF and NRAS mutations." Sci Rep. 5, 11198; doi: 10.1038/srep 11198 (2015)
5. Kakavand H, Wilmott J, Menzies A et al: "PD-L1 expression and tumor-infiltrating lymphocytes define different subsets of MAPK inhibitor-treated melanoma patients." Clin Cancer Res (2015); 21: 3140-3148
6. Jang S, Atkins M: "Which drug and when, for patients with BRAF-mutant melanoma?" Cancer Oncol (2013); 14: e60-69

INVITED LECTURES

L 10

MOLECULAR TESTING FOR LUNG CANCER: WHICH TEST FOR WHICH PATIENT?

Pr Paul Hofman, MD, PhD

Laboratory of Clinical and Experimental Pathology and Hospital-Related Biobank (0033-00025), Pasteur Hospital, University of Nice Sophia Antipolis, France

New treatment options in advanced non-small cell lung carcinoma (NSCLC) targeting activating epidermal growth factor receptor (EGFR) gene mutations and other genetic alterations demonstrated the clinical significance of the molecular features of specific subsets of lung tumors. Therefore, the development of personalized medicine has strongly stimulated the routine integration into pathology departments of different testing for somatic mutations. However, clinical mutation testing must be optimized and standardized with regard to histological profile, type of samples, pre-analytical steps, methodology and result reporting. Routine molecular testing in NSCLC is currently rapidly moving beyond EGFR mutational analysis. Thus, recent progress of targeted therapies will require molecular testing for a wide panel of mutations and for a more stratified molecular diagnosis. Novel molecular targets include for example, ALK, ROS1, BRAF and HER2 and probably soon, KRAS, c-MET, RET, PIK3CA, FGFR1 and DDR2. As a consequence, efficient testing of multiple molecular abnormalities is an urgent requirement in thoracic oncology. Moreover, increasingly limited tumor sample becomes a major challenge for molecular pathology. Continuous efforts should be made for safe, effective and specific molecular analyses and molecular testing should be always performed in accredited laboratories. This must be based on close collaboration between the different clinical and biopathology departments involved in the management of lung cancer. Moreover new challenges are coming for molecular pathologists working in lung cancer such as, 1) moving the technology to Next Generation Sequencing approach, 2) Integrating the concept and the usefulness of liquid biopsy (with both consideration for circulating tumor cell and free circulating tumor DNA detection), 3) Considering the potential use of some new antibodies which can be applied by immunohistochemical methods for a theragnosis approach and as companion tests, and, 4) Assessing at the same time both genomic alterations and new biomarkers (such as PD-L1/PD1) linked to immunotherapy. In this presentation, we will explore not only the practical issues of these new developments but also some pitfalls surrounding the routine implementation of different molecular testing in lung oncology.

L 14

KCE REPORT 219: "ORGANISATION OF CARE FOR ADULTS WITH A RARE OR COMPLEX CANCER"

S. Stordeur (KCE) , F. Vrijens (KCE), K. Henau (SKR/FRC) , V. Schillemans (SKR/FRC), C. De Gendt (SKR/FRC), R. Leroy (KCE)

Recommendation for a second opinion in pathology for rare cancers

P. Vermeulen, C. Colpaert, C. Cuvelier, P. Demetter, A. Mourin, P. Goddeeris, M. Petein, P. Delvenne, W. Waelput, R. Croes, R. Sciot, I. Salmon, T. Tousseyn, R. Achten, E. Mutijima, K. Cokelaere

Background of the KCE report

Rare cancers present a challenge to clinical practice: the experience with their diagnosis, staging and treatment is often limited, even in major cancer centres [1]. Because of the paucity of expertise, rare cancers are often diagnosed late or misdiagnosed [2]. Pathologists may be confronted with a specific rare cancer only once or twice in their entire professional career. Once the correct diagnosis is made, patients and physicians may struggle to find the information they need about the disease, how it will affect the patient and what the best treatment options are. For many rare cancers, new treatments are difficult to evaluate since it is difficult to recruit enough patients for adequately powered trials. Consequently, evidence-based clinical guidelines are seldom, if ever, available [3].

A rare cancer is defined as a cancer which affects less than 6 new adult patients/100 000 adult inhabitants/year. This threshold is based on a European definition (RARECARE) [4] and corresponds in Belgium to approximately 530 new cases per year. The two most common families of rare cancers are digestive cancers (mainly cancers of the liver and gallbladder) and haematological malignancies (mainly myeloproliferative neoplasms and acute myeloid leukaemia). The other rare cancers are spread over several families (e.g. female genital system, male genital system, head and neck). The majority of rare cancers affects less than 100 patients a year. This fact is of utmost importance when planning a better organisation of care for these patients.

The overall aim of the KCE report was to propose a coherent strategy for the management of adult patients with rare cancers and cancers that require complex care in Belgium. This objective was specifically formulated in the National Cancer Plan (2008-2010): Action 13 relates not only to the care of patients with rare cancers but also to cancers (rare or common) that require complex care including complex diagnostic and therapeutic procedures, to be carried out by highly skilled and experienced healthcare providers.

1. KCE : Centre Fédéral d'Expertise des Soins de Santé / Federaal Kenniscentrum voor de Gezondheidszorg
2. SKR/FRC : Stichting KankerRegister / Fondation Registre du Cancer

Several European countries have already adopted a differentiated model for the organisation of highly specialised cancer care, by referring adults with rare cancers to Reference Centres. The goals are universal: to raise the quality of care delivered to patients and to help patients find specialty care at facilities proven to have delivered better outcomes. The overarching point of view is that it is no longer practicable, efficient or ethical that every hospital or every practitioner continues to offer care for every rare/complex cancer. All these initiatives pursue the improvement of quality of care by reducing the dispersion of specialised care services.

Scope of this presentation

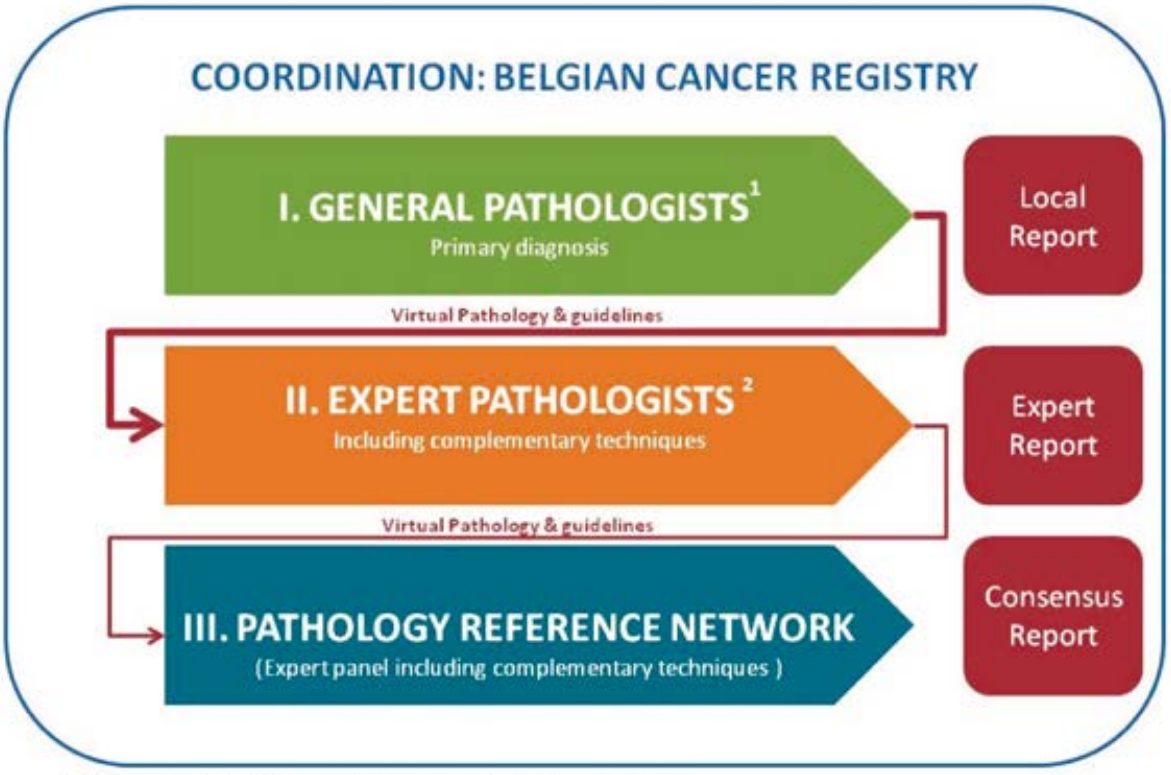
The scope of this presentation is focused on the second opinion in pathology for rare cancers.

Because the accurate diagnosis of some rare cancers presents a real challenge, a second opinion in diagnostic pathology is more and more demanded by the pathologists themselves to reduce misdiagnoses [5]. Second opinions on pathology diagnoses are routinely used intra-departmentally for a limited selection of cancer cases. Moreover for a certain number of cancer types, extra-departmental referral of cases to a panel of pathologists with acknowledged expertise in the diagnosis of certain cancer types is organised. However, at present there are no criteria to select which cases should be submitted to second opinion. Timely expert review is in the best interests of the patient, but many pathologists are faced with questions about how, when and to whom cases should be referred. Moreover, sending cases to outside institutions represents a cost for both the referring and the receiving laboratory. Currently there is neither funding of nor a legal basis for this practice of second opinion in pathology. The Belgian Institute of Public Health (WIV/ISP) is starting an external quality assurance programme for pathology laboratories, a process that deserves support.

The panel of pathologists with expertise in rare cancer diagnosis consulted in the course of the KCE study suggested a number of proposals for an improved diagnostic process of rare cancers. These proposals were unanimously accepted by the Consilium Pathologicum Belgicum, by the Commission for Anatomic Pathology, by the Belgian Society of Pathology (BWP) and by the GBS/VBS (Groupe des Unions Professionnelles Belges de Médecins Spécialistes/Verbond der Belgische Beroepsverenigingen van Geneesheren-Specialisten - Pathology). These proposals are reported hereunder, grouped into one main recommendation:

A 'three-step' model of diagnostic confirmation of pathology findings is recommended for rare cancers (Figure 1). This protocol should be implemented as recommendation of good practice in licensed pathology laboratories.

Figure 1 – A 'three-step' model of diagnostic confirmation for rare cancers



(1) General pathologists belonging to a licensed pathology laboratory
(2) Expert pathologists recognised by the ISP/WIV

Definitions

An expert pathologist is a pathologist who provides a 'second opinion' on pathology specimens from rare cancer cases. This diagnostic confirmation has to be incorporated in the initial pathology report as "expert report" within acceptable time limits. The expert pathologist works in consultation with other national and/or international expert pathologists in a 'pathology reference network' where difficult cases are discussed and a consensus diagnosis is reached.

A pathology reference network represents a panel of national and/or international expert pathologists, who will assure the second opinion of difficult cases, for a given group of rare cancers leading to a consensus report.

Practical organisation

Expert selection

Similarly to recognition process of laboratories, pathologists should apply for recognition as an expert pathologist with advice from the Commission for Pathology (ISP/WIV). To this purpose a Working Group 'quality assurance of second opinion' should be installed within the Commission.

Selection criteria will be based on 'recognition by peers', activity in relation to rare cancers (number of cases seen, taking part in multidisciplinary oncology meetings (MOC/COM), considerable daily practice in the area of expertise), scientific visibility, involvement in research and publications. Moreover, the expert pathologist has to have easy access to the necessary ancillary techniques to obtain accurate diagnosis and prognostic report on the cancers relevant to his/her area of expertise.

Pathology reference networks' composition

The pathology reference networks should be composed of a minimum number of both academic and non-academic, national and/or international pathologists. The pathology reference networks are coordinated by a responsible pathologist elected for a term of 3 years. Timing of meetings is subject to specific needs. In order to minimise delays in answering time the use of digital pathology should be introduced.

The pathology reference networks have also:

- to promote research on these rare cancers through multicentre research studies, both at a national and international level,
- to contribute to the epidemiologic surveillance of these cancers by establishing a database for collection of relevant data, in collaboration with the Belgian Cancer Registry,
- and to participate in the formulation of national recommendations for good practice, drawing on European or international guidelines.

In view of its relevance in the registration of all cancer cases, including rare cancers, the Belgian Cancer Registry would be the evident choice to coordinate this model of diagnostic confirmation of pathology data.

Daily practice second opinion organization

According to multidisciplinary oncological consultations (COM/MOC) a demand for pathological diagnostic confirmation in rare cancer cases should be addressed by the general pathologist to an expert pathologist previously defined. This expert report should be delivered in a timely manner (e.g. one week), in order to minimise any delay in treatment and has to be integrated in the initial report by the general pathologist providing a clear unique diagnosis to the clinicians. In case of discordance or for more complex cases, the expert decides to refer to the pathology reference network in order to obtain a consensus diagnosis in a timely manner.

Quality Assurance

The pathology reference networks should draft an annual activity report. This report should include (non exhaustive listing) the number of cases discussed in the panel, number of cases seen in 'second opinion' by individual expert pathologists, concordance and discordance levels, ancillary techniques used by expert pathologists. This report should be communicated to the Commission for Pathology. The ISP/WIV presents an annual composite report on the entirety of 'second opinion' activities, in accordance with the national external evaluation programme.

Virtual Pathology

The vast advancements in telecommunications and converting medical information to a digital format have increased the number of medical applications including virtual pathology. In the last few years, telepathology has benefited from the progress in the technology of image digitalization and transmission through the world web.

Virtual pathology is a rapidly evolving niche in the world of pathology and is likely to increase in popularity as technology improves. Virtual pathology facilitates rapid, efficient communication between subspecialty pathologists and generalist pathologists. This approach allows 2nd opinion on challenging cases with fine-tuning of diagnostic interpretation and has many advantages. Indeed, virtual microscopy for 2nd opinion avoids mailing costs and loss of slides. In addition, the patients will benefit from a faster diagnosis via a secure web site. Consequently, faster patient diagnosis and treatment may decrease healthcare costs.

Furthermore, an extension to the existing 'Belgian virtual tumour bank' (biobanking) could be envisioned by the possibility of digital archiving of rare cancer cases.

Financial aspects

There is an evident cost to these 'second opinions': besides logistics (transfer of slides, registration, reporting) there is an important investment in 'time and energy' of expert pathologists. No reimbursement for diagnostic confirmation of pathology data is currently provided. When considering the financial aspects of diagnostic confirmation of pathology data in rare cancers however, one should take into account other, less readily quantifiable costs. The impact on public health, patients, institutions and society of incorrect pathology diagnoses is crystal clear. A well-organised model for 'second opinions' will certainly lead to budget savings by avoiding unnecessary treatments (not to mention possible litigation costs of mistreatment).

Expert pathologists should receive a 'consultation fee' for the second opinion in the context of this programme. Coordination, secretariat and other missions of pathology reference networks could be funded through a NIHDI convention specific to this purpose.

Through the work of the Belgian Cancer Registry, in concert with many stakeholders, a very adequate estimate regarding the numbers of rare cancers can be made. These constitute only a fraction of daily pathology practice. The expected budget for these 'second opinions' would thus be very predictable and stable.

The diagnosis of these cancer cases, by their rare and complex nature, usually necessitates the extensive use of relevant ancillary techniques (e.g. immunohistochemistry, molecular biology), essential to provide a 'state of the art' reporting (fine-tuning of diagnosis, standard reporting, prognostication). It would therefore be reasonable to consider upscaling the current limit on reimbursement of these techniques.

In view of the many potential benefits of virtual pathology (especially time-wise), it could be sound financial management to fund the development of virtual pathology in Belgium, included use of digital slide-scanners, according to the existing project such as Belgian Virtual Tumour bank- Biobanking and Biomolecular Resources Research Infrastructure -Biobanking.

Finally, to manage the additional costs in general pathology labs (e.g. sending cases for double reading, registration of discordances) to be expected from this model of 'second opinion', a 'lump budget' per patient can be considered.

References

1. Gatta G, van der Zwan JM, Siesling S, Otter R, Tavilla A, Mallone S, et al. Technical report with basic indicators for rare cancers and health care related macro indicators. 2010. Grant Agreement N° 2006113; Work Package N°5; Deliverable N°13
2. European Parliament. Proceedings of the workshop: 'Rare cancers: the added value of closer cooperation'. Brussels 2011. Available from: http://www.europarl.europa.eu/RegData/etudes/divers/envi/2011/464440/IPOL-ENVI_DV(2011)464440(PAR00)_EN.pdf
3. Wagstaff A. Europe's rare cancer community calls for a more radical approach. Cancer World. 2009.
4. Gatta G, Van Der Zwan JM, Casali PG, Siesling S, Dei Tos AP, Kunkler I, et al. Rare cancers are not so rare: The rare cancer burden in Europe. European Journal of Cancer. 2011;47(17):2493-511.
5. Arbiser ZK, Folpe AL, Weiss SW. Consultative (expert) second opinions in soft tissue pathology. Analysis of problem-prone diagnostic situations. Am J Clin Pathol. 2001;116(4):473-6.

The full KCE report can be downloaded at https://kce.fgov.be/publication/report/organisation-of-care-for-adults-with-rare-cancers-and-cancers-with-complex-diagno

L 16

ETHICS AND ECONOMY: THE DUTCH EXPERIENCE.

Prof Dr P.M. Kluin, pathologist University Medical Center Groningen, Groningen, The Netherlands. Chairman committee on quality assurance and member of the board of the Dutch Society of Pathology,

In the Netherlands, pathology is practiced by pathologists who are direct employees of a hospital or of an independent foundation, whereas pathologists also can form a partnership. Historically, pathology diagnostics is financed by separate tariffs for the laboratory part and for the honorarium part. These (maximum) tariffs were secured by the Dutch Healthcare Authority (NZA). For the laboratory part this is still the case with tariffs for different techniques (cervical screening, other cytology specimen, histology, immunohistochemistry, different types of molecular analysis etc). In the past, a very simple system existed for the honorarium part of the pathologists, all histology being accredited by the same tariff that was not related to the complexity of the specimen. Since January 2015, this system has been changed, under the condition that the change should be budget – neutral at the macro-economical level . Based on the complexity of a case, there are 6 categories (for instance with different mean standard times from 7.7 to 86 minutes per case). The local financing of these tariffs has to be negotiated with the individual hospitals. An exceptional situation exists for diagnostics for general practitioners with integral maximum NZA tariffs for both the laboratory and pathologists.

Apart from the primary diagnostics, also revisions (in case a patient is referred to a second hospital and the clinicians in that hospital request a diagnosis to be reviewed) are now financed by the same system, the second hospital having this pathology review in their budget according to the "diagnosis treatment combination" (DBC or DOT) system.

Historically consultations between pathologists were not regularly financed. In case of structural collaboration between pathology departments, often local contracts were made, in particular for performing molecular diagnostics. In the new system the honorarium part is covered and (in view of the complexity of most cases) category 4 has been assigned to these consultations, but the individual laboratories are free to negotiate. In consequence highly variable individual tariffs / contracts are present at the moment, often integrating laboratory and honorarium costs.

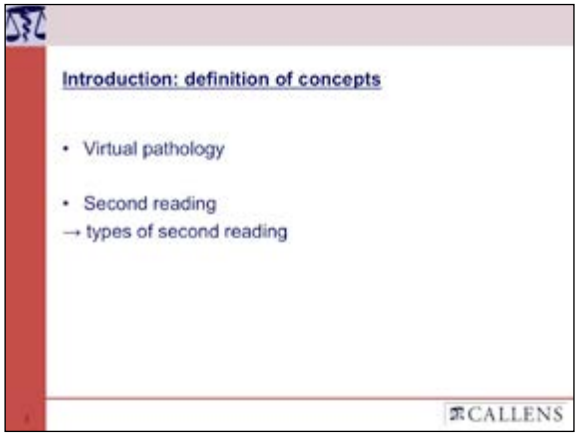
In the Netherlands regional panels exist where interested pathologists physically meet to discuss difficult or interesting cases. Some panels have also digital meetings or, more frequently, have a combination of digital preview with physical meetings. There are few national panels such as the bone tumor committee, the mesothelioma panel and the Dutch cutaneous lymphoma panel, but most panels are (supra)regional, mostly centered around the university medical centers. In the past many panels were founded by one of the 9 regional comprehensive cancer centers. In consequence the individual panels were variably financed (from nil to financing honorarium and even some lab costs) by these cancer centers. Since 2014, one national comprehensive cancer center, the IKNL, exists

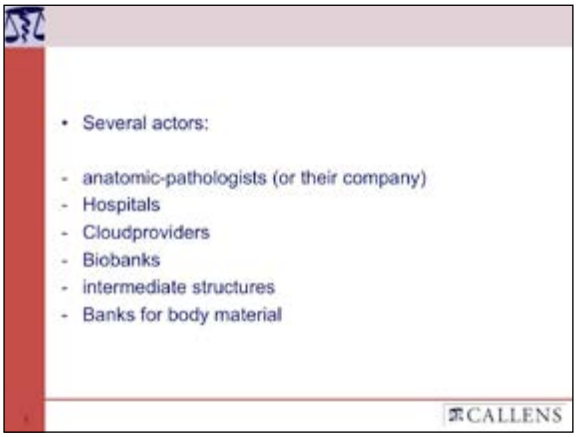
and 13 panels (malignant lymphomas, melanomas and soft issue tumours) are centrally co-financed by this institute. The money can be used for administration or compensation of travelling costs, but not for lab costs etc. If a case needs extra work up it should be considered as a consultation and financed as such.

At present the Dutch Society of Pathology and the pathological anatomy national automated archive PALGA are working on a unique new national digital platform in which (locally or centrally stored) digital images of specimen can be integrated with the centrally stored pathology reports. This system will be co-financed by IKNL. This platform will be a very powerful tool for inter-laboratory consultation but also for national and regional panels.

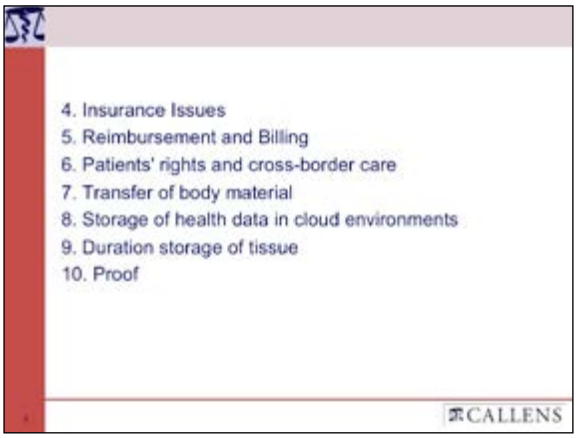
L 18

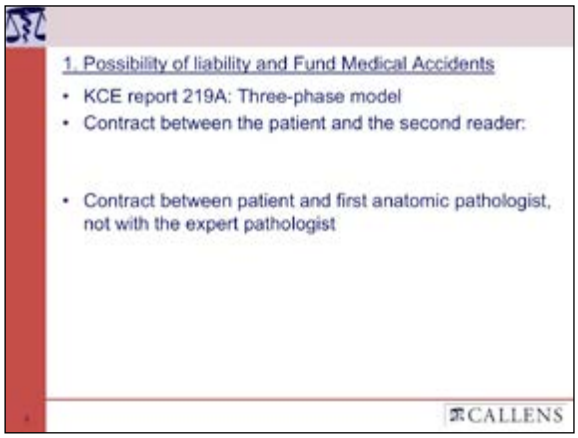

Legal aspects of second opinion pathology and/or virtual pathology
Stefaan Callens
CALLENS


Introduction: definition of concepts
• Virtual pathology
• Second reading
→ types of second reading
CALLENS

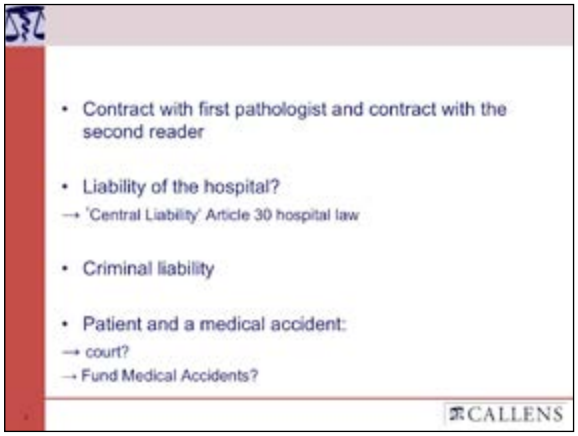

• Several actors:
- anatomic-pathologists (or their company)
- Hospitals
- Cloudproviders
- Biobanks
- intermediate structures
- Banks for body material
CALLENS

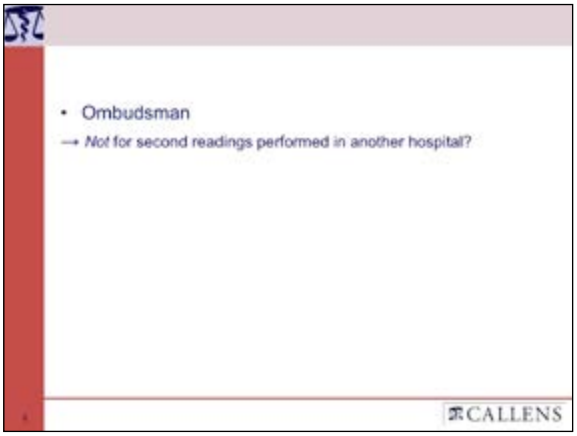

Overview of legal aspects
1. Liability and the Fund Medical Accidents
2. Competent court and applicable law in case of international second opinion
3. Cooperation agreements with other institutions
CALLENS

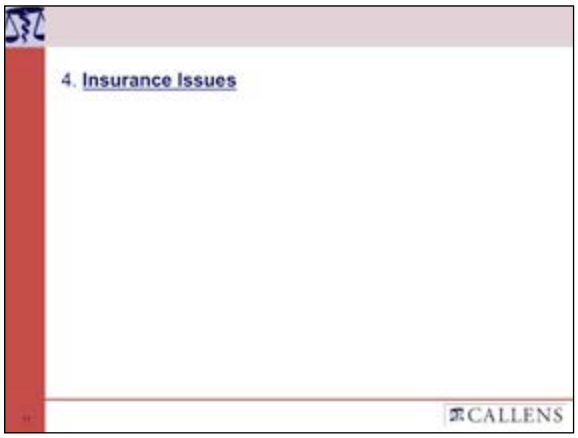

4. Insurance Issues
5. Reimbursement and Billing
6. Patients' rights and cross-border care
7. Transfer of body material
8. Storage of health data in cloud environments
9. Duration storage of tissue
10. Proof
CALLENS

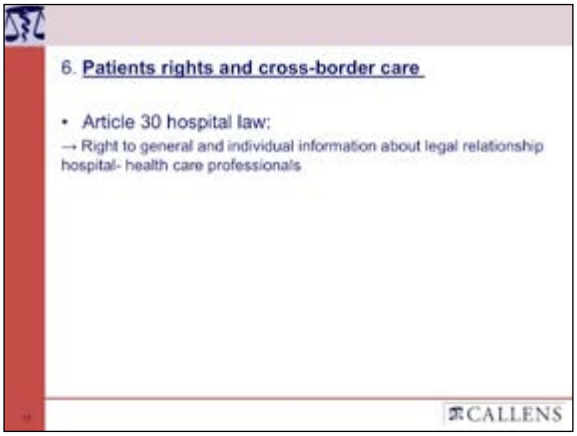

1. Possibility of liability and Fund Medical Accidents
• KCE report 219A: Three-phase model
• Contract between the patient and the second reader:
• Contract between patient and first anatomic pathologist, not with the expert pathologist
CALLENS

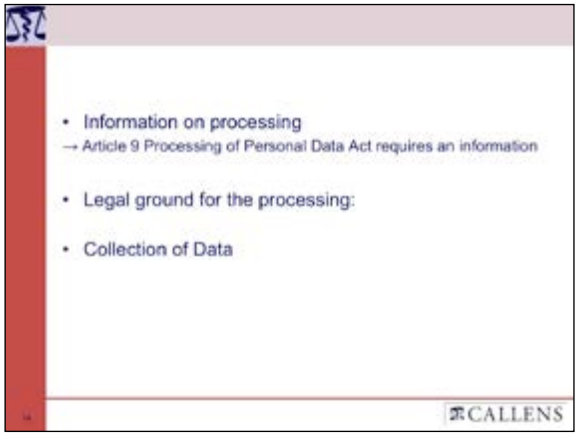
INVITED LECTURES

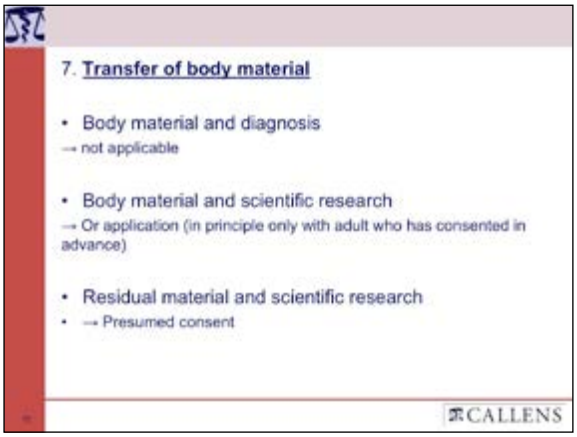


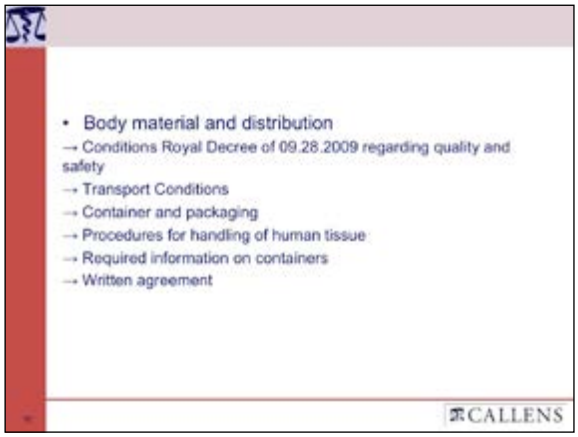


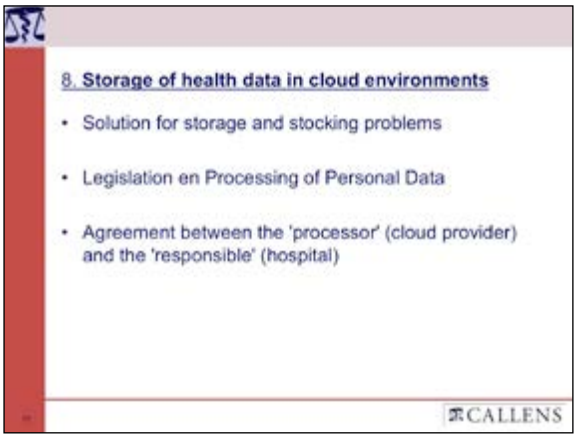


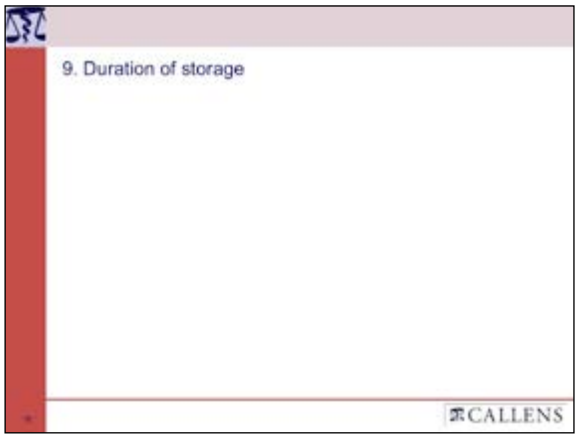

6. Patients rights and cross-border care
• Article 30 hospital law:
→ Right to general and individual information about legal relationship hospital- health care professionals
CALLENS


• Information on processing
→ Article 9 Processing of Personal Data Act requires an information
• Legal ground for the processing:
• Collection of Data
CALLENS

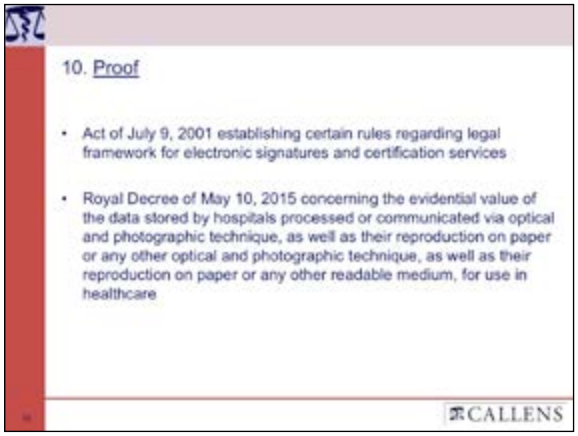

7. Transfer of body material
• Body material and diagnosis
→ not applicable
• Body material and scientific research
→ Or application (in principle only with adult who has consented in advance)
• Residual material and scientific research
• → Presumed consent
CALLENS


• Body material and distribution
→ Conditions Royal Decree of 09.28.2009 regarding quality and safety
→ Transport Conditions
→ Container and packaging
→ Procedures for handling of human tissue
→ Required information on containers
→ Written agreement
CALLENS


8. Storage of health data in cloud environments
• Solution for storage and stocking problems
• Legislation en Processing of Personal Data
• Agreement between the 'processor' (cloud provider) and the 'responsible' (hospital)
CALLENS


9. Duration of storage
CALLENS

INVITED LECTURES



L 19

ROLE OF THE PATHOLOGIST IN THE DIAGNOSIS OF INFECTIOUS DISEASES

Pr Paul Hofman, MD, PhD
Laboratory of Clinical and Experimental Pathology and Hospital-Related Biobank (0033-00025), Pasteur Hospital, University of Nice Sophia Antipolis, France

Currently there exists a paradox between the frequency of diagnosis of lesions caused by pathogens in a pathology laboratory and the gradual loss of expertise of clinical pathologists in the field of infectious and tropical diseases. A number of reasons could explain this situation, including, 1) The omnipresence of tumour samples to be managed by the pathologists and the new and additional constraints related to the use of many targeted therapies, which require the mandatory assessment of new biomarkers, 2) The strong necessity that surgical and molecular pathologists hold suitable competence in both pathology/cytopathology and microbiology when working in the field of infectious and tropical diseases pathology, 3) The expertise in infectious diseases pathology requires the understanding of the taxonomy and the classifications of pathogens as well as a good knowledge of the morphological criteria that allow their identification, and, 4) The increasingly important role of the microbiology laboratories in diagnosing rapidly and accurately infectious diseases. This presentation highlights the current and future issues pertaining to the pathology of infectious and tropical diseases for the clinical and molecular pathologists. We will present the potential usefulness of different current and future ancillary methods which can be developed in a pathology laboratory for the improvement of infectious diseases diagnosis.

INVITED LECTURES

L 20

SALIVARY GLANDS.

Jerzy Klijanienko MD PhD, MIAC

Salivary gland classification undergoes constant modifications. Recently, few new tumor entities were described which will be included in a new WHO classification. Moreover, characteristic and specific molecular modifications were also been indentified, which may help in the diagnosis.

In routine practice, salivary gland tumors are usually diagnosed on frozen sections followed by surgical excision. As an alternative way, in many centers a pre-operative cytological diagnosis is more accurate than diagnosis from frozen sections. The cytologic diagnosis is based on discrimination between tumoral/pseudo-tumoral/inflammatory, malignant/benign, epithelial/non-epithelial, high-grade/low-grade tumor typing. Moreover, cytology material may be successfully used for immunohistochemistry and molecular techniques.

Recently we have proposed a cytological classification of salivary lesions according to a predominant cytological tumor component. Lesions were classified into myoepithelial, oncocytic, basal, acinic, squamous, and non-specific groups of predominant cellular pattern. Importance of stroma and secretions were also highlighted. We have shown a high specificity and sensitivity of this technique. However, cellular material was successfully used for ancillary techniques.

In conclusion, the combination of cytology, frozen sections and molecular data is optimal and correlative method in histological diagnosis in salivary gland lesions.

L 21

HEAD AND NECK CARCINOMA AND HPV

Belén Lloveras, M.D., Ph.D.

Head and neck cancer incidence, traditionally not very high in Western countries, has increased even though smoking habits have dropped. This type of cancer has been classically attributed to tobacco and alcohol consumption but in recent years, a subset of these tumors has been related to Human papillomavirus (HPV) infection, with a very variable proportion according to geography. The precise location of tumors that have depicted a significant increase gives some clues on which are the sites that are most frequently related to HPV infection. While laryngeal, floor of the mouth and gum cancers have decreased, oropharyngeal cancers are on the rise, and these are the ones with higher proportion of HPV. Several epidemiological studies have shown that there is enough statistical support to involve HPV 16 in oropharyngeal cancers and in 2007 the International Agency for Cancer Research declared it as a carcinogen for cancer of this anatomical site. However, the study of the etiological involvement of HPVs in head and neck carcinogenesis is challenged by its multifactorial etiology and data are still controversial particularly on clinical grounds when decisions on patient management need to be taken. Molecular studies have described a larger number of chromosomal aberrations in tumors caused by tobacco than in HPV related and the mere detection of HPV DNA in tumor samples may reflect a passive infection or contamination and therefore be insufficient to prove its role in etiopathogenesis. It has been demonstrated that clinical behavior is different depending on the carcinogenic trigger of the tumor and new oncology treatments are being developed for the less aggressive HPV related cancers, that present an overall 5 years survival of 80% even at advanced stages. It is of note that for the same T status usually HPV-related tumors present with a more advanced N stage, and frequently the lymph nodes are cystic. It seems that the HPV tumors might be more radiotherapy sensitive. For this reason markers of viral activity, not just HPV DNA, are requested (mRNA, p16 expression) to define a tumor as HPV-driven.

The WHO recognizes several histologic subtypes of head and neck carcinomas and some of them (basaloid, non-keratinizing) are often caused by HPV. However, there is not a perfect correlation between morphology and HPV DNA detection, some basaloid tumors may not harbor HPV DNA while other squamous carcinomas with no basaloid characteristics can be HPV related. In routine pathology there is a need for molecular tests to prove the HPV etiology because of its clinical impact. Presently, there is still no consensus on which should be the markers in routine clinical practice to determine if a tumor is HPV linked.

In the Catalan Insitute of Oncology we conducted a study over 4000 paraffin blocks corresponding to head and neck cancers from 29 different countries worldwide, with an overepresentation of Europe and Central-South America. Different immunohistochemical markers (p16INK4a, pRb, p53, and Cyclin D1) were evaluated against the gold standard that was considered a positive result with highly sensitive techniques of both HPV DNA and RNA in tumor tissue. Samples from larynx, oropharynx and oral cavity showed different results. Squamous cell carcinomas from oropharynx, in particular from the tonsil showed the highest HPV prevalence (25%) by all methods while carcinomas from the oral cavity (7.4%) and larynx (5.9%) the lowest. In general, the HPV attributable fraction was lower when markers of active HPV infection were included in the analysis dropping to 22%, 4% and 3% for the cited anatomical sites. The immunohistochemical marker that appeared as more reliable for active HPV infection was the overexpression of the cell cycle associated protein p16INK4a.

Bibliography

1. A review of human carcinogens. Part B: Biological agents / IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2009: Lyon, France).
2. IARC Monographs on the evaluation of carcinogenic risks to humans, vol. 83. Tobacco Smoke and Involuntary Smoking. Lyon, France: International Agency for Research on Cancer; 2004.
3. IARC Monographs on the evaluation of carcinogenic risks to humans, vol. 44. Alcohol drinking. Lyon, France: International Agency for Research on Cancer; 1988.
4. Herrero R, Castellsagué X, Pawlita M, et al; IARC Multicenter Oral Cancer Study Group. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 2003;95:1772-83.
5. Holzinger D, Schmitt M, Dyckhoff G, Benner A, Pawlita M, Bosch FX. Viral RNA patterns and high viral load reliably define oropharynx carcinomas with active HPV16 involvement. Cancer Res 2012;72:4993-5003.
6. Ndiaye C, Mena M, Alemany L, et al. HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. Lancet Oncol 2014;15:1319-31

7. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005;14:467-75.
8. Barnes L, Eveson JW, Reichart P, Sidransky D (Eds.): World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. IARC Press: Lyon 2005.
9. Smeets SJ, Hesselink AT, Speel EJ, et al. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. Int J Cancer 2007;121:2465-72.
10. Robinson M, Schache A, Sloan P, Thavaraj S. HPV specific testing: a requirement for oropharyngeal squamous cell carcinoma patients. Head Neck Pathol 2012;6: S83–90.
11. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. J Clin Oncol 2013;31:4550-9.
12. Mehanna H, Beech T, Nicholson T, et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer--systematic review and meta-analysis of trends by time and region. Head Neck 2013;35:747-55.
13. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011;29:4294-301.

INVITED LECTURES

L 22

ODONTOGENIC CYSTS; AN OVERVIEW WITH EMPHASIS ON THE HISTOPATHOLOGICAL ASPECTS

Isaäc van der Waal, Amsterdam

Odontogenic cysts are derived from epithelium of the odontogenic apparatus. Some of these cysts are caused by an inflammatory stimulus (inflammatory cysts) while for other cysts such a stimulus is unknown, being referred to as developmental cysts (Table I; slightly modified from the WHO classification 1992).

Table I Classification of odontogenic cysts (slightly modified from the WHO classification 1992)*

1 Developmental cysts
1.1 Gingival cyst of the newborn ("Dental lamina cyst")
1.2 Gingival cyst of adults
1.3 Eruption cyst
1.4 Follicular (dentigerous) cyst
1.5 Lateral periodontal cyst
1.6 Keratinizing odontogenic cyst
1.7 Sialo-odontogenic cyst ('Glandular odontogenic cyst')
2 Inflammatory cysts
2.1 Radicular cyst
2.2 Residual (radicular) cyst
2.3 Paradental cyst (including the mandibular buccal infected cyst)

* The lesion previously known as odontogenic keratocyst is currently designated "Keratocystic odontogenic tumor", being classified as an odontogenic tumor.

The majority of the odontogenic cyst is llocated within the jaw bones (intraosseous), while odontogenic cysts in the soft tissues (peripheral) are rare, indeed. Odontogenic cysts may become symptomatic but may also be detected as an incidental finding on a radiograph. Malignant changes in the epithelium of odontogenic cysts is exceedingly rare. Odontogenic cysts are treated by enucleation or, occasionally, by marsupialization.

A few odontogenic tumors may present as a cyst, e.g. the unicystic ameloblastoma and the keratocystic odontogenic tumor ("odontogenic keratocyst"). These tumors will be briefly discussed as well.

For proper communication with the clinician the pathologist should be familiar with the nomenclature of the dentition (Table II).

Tabel II Nomenclature of the teeth

Deciduous dentition		
maxilla		
	55 54 53 52 51	61 62 63 64 65
right		left
	85 84 83 82 81	71 72 73 74 75
mandible		

Permanent dentiton		
maxilla		
	18 17 16 15 14 13 12 11	21 22 23 24 25 26 27 28
right		left
	48 47 46 45 44 43 42 41	31 32 33 34 35 36 37 38
Mandible		

Note: 11 (upper central incisor in the right part of the maxilla) should be pronounced as one-one

1 Odontogenic cysts

1.1 Developmental odontogenic cysts

1.1.1 Gingival cyst of the newborn ("Dental lamina cyst of the newborn")

Developmental cyst located in the mucosa of the alveolar ridge of a newborn. Presents as solitary or multiple whitish-greyish nodules on the upper or lower alveolar ridge. The diagnosis is usually a clinical one; only in rare instances the taking of a biopsy may be considered. The lining consists of stratified squamous epithelium. The cyst lumen is filled with keratin. The cyst(s) will be disappear spontaneously in a matter of weeks.

1.1.2 Gingival cyst of adults

Odontogenic developmental cyst in the gingiva of an adult. Presents as a cystic swelling of the gingiva. The gingival cyst is asymptomatic otherwise.

Histopathologicaaly a single or double layered epithelial lining and occasionally formation of epithelial "plaques" will be observed. Treatment consists of excision; the cyst rarely recurs.

1.1.3 Eruption cyst

Developmental cyst, that can be regarded as a follicular cyst of an erupting tooth. The clinical presentation is a bluish, cystic lesion on the alveolar ridge at the site of an erupting tooth, usually a deciduous molar in the upper jaw. The diagnosis can usually be made on clinical grounds alone. A biopsy is only indicated in case of doubt. The epithelial lining consists of stratified squamous epithelium.

Treatment is not required. The underlying tooth will erupt in a normal way.

1.1.4 Follicular (dentigerous) cyst

Cyst development in a follicle of an unerupted tooth. It is a quite common cyst that almost exclusively occurs in the permanent dentition. The cyst is usually detected as an incidental finding on a radiograph, often being related to mandibular and maxillary wisdom teeth and upper canines.

Radiographically, a well-defined radiolucency around the crown of an impacted tooth is observed. The differential diagnosis includes, among others, a keratocystic odontogenic tumor and an ameloblastoma.

The lining of a follicular cyst consists of two or more layers of squamous epithelium without any characteristic features. Occasional "goblet cells" may be observed. Histopathologically, no distinction can be made between the lining of a follicular cyst and the lining of an (enlarged) tooth follicle. In tooth follicles embryonal rests of odontogenic epithelium may be encountered, sometimes mimicking ameloblastomatous cells. Furthermore, myxoid changes in the stroma can be observed, mimicking to some extent the features of an odontogenic myxoma. Treatment consists of enucleation and removal of the associated tooth.

1.1.5 Lateral periodontal cyst

In the past, a lateral periodontal cysts has been defined clinicoradiographically as a cyst located between the roots of two vital teeth. At present, the definition has been extended by certain histopathological characteristics. The lateral periodontal cyst is almost exclusively found in the premolar area of the mandible, usually being an accidental finding on a radiograph.

The histopathology shows an epithelial lining of just a few cell layers thick with occasionally characteristic epithelial plaques. In fact, a more or less similar lining is seen in the gingival cyst of the adult, as has been discussed before.

Treatment consists of enucleation; recurrence is rare.

1.1.6 Keratinizing odontogenic cyst

The keratinizing odontogenic cyst (KOC) is a developmental odontogenic cyst, presenting clinicoradiographically as a follicular cyst. The diagnosis is based on histopathologic features only. These features consist of an epithelial lining showing hyperorthokeratosis without the characteristic epithelial architecture of a keratocystic odontogenic tumor (KCOT). In the past, this cyst was regarded as the orthokeratotic variant of a KCOT, that, in contrast to the parakeratotic variant of a KCOT, rarely recurred after removal.

1.1.7 Sialo-odontogenic cyst

Rare developmental cyst with tubular-like structures in the epithelial lining which resemble salivary gland tissue. Usually located in the anterior part of the lower jaw. Radiologically, the cyst presents as a unilobular or multilobular radiolucency, being indistinguishable from a keratocystic odontogenic tumor or ameloblastoma.

Apart from squamous epithelium also tubular structures and mucous producing cells are encountered that can somewhat resemble mucoepidermoid carcinoma. Treatment consists of enucleation.

1.2 Inflammatory odontogenic cysts

1.2.1 Radicular cyst

Cyst around the apex of a root (some prefer the term "periapical" cyst) of a non-vital tooth. Rarely occurs in the temporary dentition. Occasionally causes a swelling or an abscess. The radiographic picture consists of a well-defined radiolucency around the apex of a root.

Histopathologically a non-characteristic lining of stratified squamous epithelium is encountered. Treatment consists of endodontic treatment, sometimes in combination with an apicoectomy. If necessary extraction of the tooth and enucleation of the cyst may be considered.

1.2.2 Residual (radicular) cyst

(Radicular) cyst left behind after a tooth extraction. A residual cyst may give rise to a swelling of the jaw. Radiologically, a well-defined radiolucent lesion is seen in an edentulous part of lower or upper jaw. The differential diagnosis includes, a.o. ameloblastoma, keratocystic odontogenic tumor and other non-odontogenic tumors.

Histopathologically, a non-characteristic lining with squamous epithelium will be observed. Occasional presence of calcifications in the lining epithelial cells, called "Rushton bodies" These Rushton bodies may be misinterpreted as part of a cystic calcifying odontogenic tumor, a rare odontogenic tumor that will not be discussed here any further.

Treatment consists of enucleation or, if indicated, marsupialization.

1.2.3 Paradental cyst (incl. mandibular buccal infected cyst)

Cyst formation in a persisting follicle of an erupted tooth; the mandibular buccal infected cyst is a rare subtype. Usually incidental finding on a radiograph. Occasionally pocket formation and mild symptoms of gingival discharge of fluid.

The paradental cysts presents radiologically as a circumscribed radiolucency, distally of an erupted tooth, often a lower wisdom tooth. The mandibular buccal infected cyst may be accompanied by a periapical radiolucency around a vital tooth.

Histopathologically, a non-characteristic epithelial lining will be observed.

Treatment consist of enucleation

2 Some odontogenic tumors that may present as a cyst

2.1 Ameloblastoma

Nearly always histologically benign tumor consisting of ameloblastlike epithelial cells.

Is characterized by a high rate of recurrence. Histopathological malignant characteristics such as cellular or nuclear polymorphism, mitotic activity and invasive growth, are extremely rare. A histologically benign ameloblastoma may in rare instances metastasize to the cervical lymph nodes or to sites elsewhere in the body, being referred to as "metastasizing ameloblastoma".

There are no known etiological factors. The estimated incidence is 1 per million population per year. Sometimes diagnosed at a young age. The average age at the time of diagnosis is around 30 years. The tumor nearly always develops in the jaw bone (intraosseous ameloblastoma), probably from residual epithelium of the dental lamina. Occasionally the tumor arises from odontogenic epithelium of the alveolar mucosa (peripheral ameloblastoma).

Radiologically, an ameloblastoma will present as a unilobular or multilobular lucency. May mimic, a.o. a residual cyst, a follicular cyst or a keratocystic odontogenic tumor. Additional CT- scans are required in order to get better information about the extent of the tumor and the integrity of the cortical plates of the jaw bones.

Most ameloblastomas are histopathologically easy to diagnose, especially in case of the common follicular type. The unicystic ameloblastoma is a rare variant, being characterized macroscopically as a cystic lesion and microscopically by a lining of ameloblastomatous epithelial cells. The diagnosis "unicystic ameloblastoma" can not be made on the basis of radiographic aspects.

Treatment consists preferably of wide surgical excision with a margin of surrounding clinically normal bone, if necessary sacrifying the continuity of the mandible. Only in case of complete, intact enucleation of a unicystic ameloblastoma, additional radical surgery can be avoided.

2.2 Keratocystic odontogenic tumor ("Keratocyst")

The diagnosis keratocystic odontogenic tumor (KCOT) is based exclusively on histopathological criteria. In the past, the lesion was classified as an odontogenic cyst. Worldwide there is an ongoing debate among (oral) pathologists about the correctness of "upgrading" the odontogenic keratocyst to a keratocystic odontogenic tumor as has been done in the WHO classification (2005).

The radiographic aspect is not pathognomonic and may resemble a.o. a residual cyst, an ameloblastoma, a central giant cell lesion and other odontogenic and non-odontogenic lesions. Multiple KCOTs, not necessarily present simultaneously, are indicative of the hereditary basal cell nevus syndrome (Gorlin syndrome). The other aspects of this syndrome are, a.o., the presence of one or more bifid ribs, pits in the skin of the hand palms, calcifications of the falx cerebri, and, most important, multiple basal cell carcinomas of the skin, often located in the head and neck area and occurring already at an early age.

A KCOT has characteristic histopathological aspects consisting, amongst others, of an epithelial lining of 6-8 cell layers thick, palisade arrangement of the basal cells and a parakeratotic, often corrugated, surface. Histopathologically, no distinction can be made between a sporadic KCOT and a KCOT in a patient suffering from the Gorlin syndrome. The characteristic features of a KCOT may disappear when the cyst becomes secondary inflamed. In case of distinct hyperkeratosis one is most likely dealing with a keratinizing odontogenic cyst (see before).

Treatment consists of enucleation and, if possible, removal of the overlying mucosa. In some cases marsupialization or decompression is to be preferred, usually requiring enucleation at a later stage. Some clinicians use the "fixation before and/or after enucleation technique", applying Carnoy's fixative solution peroperatively and/or after enucleation.

The recurrence rate of KCOTs depends on the extent of the surgical removal and may be up to 25% or more during a follow-up period of five years after just enucleation.

In case of suspicion of the basal cell nevus syndrome, referral to a department of clinical genetics is indicated.

References:

1. Kramer IRH, Pindborg JJ, Shear M. WHO International Histological Classification of Tumours, 2nd ed., Springer-Verlag, Berlin Heidelberg New York, 1992.
2. Barnes L, Eveson JW, Reichart P, Sidransky D. WHO Classification of Tumours. Pathology and genetics. Head and Nrck Tumours. Lyon: International Agency for Reasearch on Cancer, 2005.

dr. Isaäc van der Waal

Professor of Oral Pathology

VU medical center

Dept. of Oral and Maxillofacial Surgery/Pathology

P.O. Box 7057

1007 MB, Amsterdam

Tel. +31-20-4441023

i.vanderwaal@vumc.nl

L 24

ORIGIN AND SIGNIFICANCE OF MACROVESICULAR AND MICROVESICULAR STEATOSIS

N. Nagy CHU Charleroi, A. Pathogenesis

Steatosis is known as accumulation of triglyceride droplets within hepatocytes. To store excess lipids, such as sterol or fatty acids, cells esterify these lipids, forming non polar neutral lipids, and package them into cytosolic lipid droplets. Liver stellate cells contain prominent retinol.[1]

Under normal metabolic conditions, hepatic fat should not exceed 5% of total liver weight. The liver does not normally store lipid, in the normal liver lipid biosynthesis is fundamental for the maintain of cellular membrane, support hepatic bile secretion which contains bile salts, phospholipids and cholesterol and play a key role in the assembly of very low-density lipoprotein particles (VLDL).[2]

The accumulation of lipid droplets is a metabolic event; the liver metabolizes excessive nutrient storage in the form of glycogen and lipids and supplies energy-producing substrates to the peripheral tissues to maintain their function.

Alteration of the hepatic metabolic balance (alcohol consumption, excessive food intake, drug…) can induce fatty liver disease, a major cause of chronic liver disease. Altered hepatic metabolism and tissue remodeling in fatty liver disease further disrupted hepatic oxygen homeostasis resulting in severe hypoxia. Thus hepatic steatosis is a key step in the development and progression of chronic pathology.

In hepatic steatosis, five major pathways determine liver fat volume: The uptake of free fatty acids and triglycerides from the diet, de novo lipogenesis, fatty acid oxidation, the export of triglyceride as VLDL into the bloodstream and the flux of fatty acids released from adipose tissue through lipolysis.

In the case of Alcoholic Fatty Liver Disease (AFLD), the accumulation of lipids is mainly due to de novo lipogenesis and impaired FA oxidation. In Non Alcoholic Fatty Liver Disease (NAFLD), adipose tissue lipolysis and hepatic de novo lipogenesis account for 59% and 26% of fat accumulation. Obstructive sleep apnea, associated with intermittent hypoxia, is another risk factor for the development of NAFLD, independent of obesity.[1]

Studies in human and rodents revealed several major regulators of lipid metabolism:

- The sterol response elements binding protein (SREBP), transcriptor factor that control de novo lipogenesis

- Lipogenic genes such as SCD-1 which is still controversial

- Peroxisome proliferator-activated receptor (PPAR) which play a role in impaired FA oxidation

- Carbohydrate response element binding protein (ChREBP)

- X-box binding protein

- Hypoxia inducible factors (HIFs) which regulate the cellular and tissue adaptive response to hypoxia which is associated with lipid metabolism.

Hypoxia is highly associated with lipid homeostasis when ischemic and hypoxic stress increase cellular lipid deposition, hypoxia also upregulates genes involved in lipogenesis, lipid uptake and lipid droplet formation.

Depletion of proteins that change the phospholipid content, dramatically changes lipid droplets morphology. Some proteins bind lipid droplets surface and regulate lipid droplets size and number, these include the PAT proteins (perilipin), CIDE proteins (Cell Death Inducing DNA Fragmentation Factor) and several lipases. [2,3,4]

B. Alcoholic disease

Alcohol abuse represents the world's third largest risk factor for disease and disability. After C virus-related hepatitis, alcohol represents the common cause of chronic liver disease in the majority of the industrialized countries. Overall, about 25% of the cases of liver cirrhosis are due as initial trigger to over-exposition to alcohol. Duration and amounts of alcohol ingested remain the most important risk factor for the development of a chronic alcoholic liver disease. It has been reported that a daily consumption of 60-80g/d of alcohol for 10 years or longer in men and 20 g/d in women leads to an advanced form of liver disease in < 40% of the cases.

Female sex, obesity, non-sex-linked genetic factors and cigarette smoking can modulate the host susceptibility to develop ALD and contribute to overall risk of developing the severe form of ALD. Alcohol also synergistically interacts with other causative agents of liver diseases such as virus B or C hepatitis, human immunodeficiency virus infection, NAFLD, hemochromatosis.[5]

B.1. Ethanol metabolism and cell injury

Liver is the main organ responsible for metabolizing alcohol consumption. Ethanol and its bioactive products, acetaldehyde-acetate, fatty acid ethanol esters, ethanol-protein adducts, have been regarded as hepatotoxins that directly and indirectly exert their toxic effects on liver. Hepatic metabolism of ethanol proceeds via oxidative and non-oxidative pathways.

Oxidative pathway: the main step is mediated by alcohol dehydrogenase and acetate dehydrogenase that transform respectively ethanol to acetaldehyde and acetaldehyde to acetate; the end products of this reaction are acetaldehyde, acetate and high levels of NADPH. Acetaldehyde damages liver by direct triggering inflammation, extracellular matrix remodeling and fibrogenesis. Furthermore, acetaldehyde covalently binds to proteins and DNA, leading to the production of immunogenic adducts (malondialdehyde) in the hepatocytes. Finally, it stimulates transforming growth factor (TGF)-beta, signaling in hepatic stellate cells which acquire a pro-fibrogenic and pro-inflammatory profiles. Electrons from alcohol are transferred to NADP+ by ADH. Changes in the ratio NADH/NAD+ may affect biochemical reactions in the mitochondria and gene expression in nucleus. The burn of NADPH requires additional oxygen amounts in the mitochondria, but despite a more uptake from arterial blood, it is not enough to adequately supply all liver regions, resulting in significant hypoxia of the perivenous hepatocytes.

Ethanol metabolism is also responsible for the generation of reactive oxygen species (ROS). Indeed, cytochrome P4502E1 (CYP2E1) is up-regulated in chronic alcohol abuse and assists ADH in converting alcohol to acetaldehyde. ROS causes oxidative stress, endoplasmic reticulum (ER) stress and steatosis. The tree main sources of ROS are mitochondria (through their respiratory chain), ER (through CYP2E1) and Kupffer cells (through NADPH oxidase). ROS are responsible for the pro-inflammatory profile of alcohol-related liver injury by: activating redox-sensitive transcription factors (nuclear factor kappa B (NF-KB)), recruiting neutrophils and other immune cells, increasing the level of circulating pro-inflammatory cytokines and contributing to the lipid peroxidation associated with alcoholic liver injury. Chronic exposure to ethanol induces glutathione depletion, which makes hepatocytes more sensitive to oxidative stress. Iron is also involved in oxidative stress and promotes fibrosis by catalyzing the formation of ROS. The cytotoxic effects of ethanol metabolism and ROS lead to cell death through apoptosis and necrosis, both present in ALD.

Non oxidative pathway: catalase, a peroxysomal enzyme, is the master regulator with the final product FAEE responsible for alcoholic steatosis, and is a useful biomarker of chronic alcohol consumption.

Gut-derived lipopolysaccharide (LPS) is also a critical trigger of liver steatosis, inflammation and fibrosis. In normal conditions, LPS, derived from Gram-negative bacteria of the gut, enters the portal circulation only in traces to be cleared by the Kupffer cells and hepatocytes that possess different LPS recognition system. Alcohol abuse impairs intestinal barrier, leading to accumulation of circulating endotoxin which bind to the CD14 surface receptor on the Kupffer cells via LPS-binding protein. The CD14-LPBLPS complex produces ROS via NADPH oxidase. ROS stimulate the Toll-like receptor 4 signaling cascade with, as end, results the activation of NF-KB and the release of inflammatory cytokines (TNF-α). TNF-α, in turn, sustains liver injury by worsening the gut permeability and upholding the necro-inflammatory hepatic damages.[5,6,7]

B.2. Alcohol-induced steatosis

Alcohol abuse increases the ratio NADH/NAD+ in hepatocytes, which disrupts mitochondrial β-oxidation of fatty acids, and results in steatosis. In alcohol abuse, an increase of lipids from the small intestine is also shown, increasing mobilization of fatty acids from adipose tissues and increasing uptake of fatty acids by the liver.[6]

Autophagy plays also an important role in removing.

Chronic alcohol abuse promotes steatosis by disrupting lipid metabolism via SREBPs and PPAR-α, which are directly influenced by AMPK (5'adenosine monophosphate-activated protein kinase). SREBP1c is responsible of increasing fatty acid biosynthesis through fatty acid synthase and enzymes responsible for fatty acid desaturation. SREBP expression is increased by acetaldehyde and TNF also responsible for stimulating its maturation. Conversely, PPAR-α, which prevents ethanol induced steatosis, is reduced by acetaldehyde, and in chronic alcohol abuse by down regulation of RXR-α which alters PPAR-α signaling.[7]

Insulin plays also a key role in steatosis due to alcohol abuse. A faster disease progression is seen in patients who are overweighed compare to normal weight patients.[7]

The frequency of alcoholic liver disease, including alcoholic steatosis, the age and severity of presentation vary by ethnicity.[8]

B.3. Histology of alcoholic liver disease

The liver involvement in ALD classically ranges from alcoholic steatosis, alcoholic hepatitis or steatohepatitis, alcoholic cirrhosis and even hepatocellular carcinoma.

Alcoholic steatosis: steatosis is defined as the accumulation of lipid droplets in the hepatocyte cytoplasm. The term fatty degeneration is used when > 5´% of hepatocytes is steatotic, while fatty liver is used when > 50 % of hepatocytes shows steatosis. Presence of steatosis is not essential for the diagnosis of ALD as the steatosis may even decrease despite continuing alcohol abuse and progression of the disease.

In ALD steatosis, the lipid droplets accumulate within hours of major alcohol abuse. ALD shows macro and microvesicular steatosis. Impaired export of VLDL leads to the accumulation of lipoprotein lipid within a distant Golgi apparatus, regardless location; these droplets initially are only a micron or two in diameter known as microvesicular steatosis. This microvesicular steatosis is characterized by multiple fat droplets with a central nucleus. Pure microvesicular steatosis may be seen in alcoholic foamy degeneration. These droplets are able to rapid dissolution through the action of the lipases acting at the cytoplasmic interface. Over time (probably days), when alcohol abuse continue, in the zone 3 progressing to the zone 1, the micro fat droplets coalesce to macro droplets exceeding 20 microns, displacing the hepatocyte nucleus. This macrovesicular steatosis has a high surface/volume ratio and is less susceptible to the action of the lipases. This macrovesicular steatosis can thus remain for months even in absence of further alcohol exposure. Finely macrovesicular fat droplets can coalesce to form fat cysts characterized by irregular extracellular fat vacuole. Continuing fat accumulation may lead to rupture of fat cysts with a histiocytic reaction or lipogranuloma.[2,9]

Alcoholic steatohepatitis (ASH) is defined by an international consensus group as evidence of hepatic injury accompanying steatosis and polymorphonuclear infiltration. Additional features of Mallory Denk bodies and cholestasis are observed but not necessary for diagnosis.

Injury is mostly characterized by hepatocyte ballooning and apoptosis.

Lobular neutrophil rich inflammation is characterized by micro abscesses and neutrophils surrounding ballooned hepatocytes called « satellitosis ». Portal inflammation is usually milder and is more common in higher grade of ALD, sometimes accompanied by ductular reaction and periportal fibrosis.

Mallory bodies are clump or skeins of eosonophilic ropy material located in the perinuclear cytoplasmic region; this eosinophilic material is made of misfold and aggregated keratin filaments.

Other histologic features of ALD include glycogenated nuclei, megamitochondria, hemosiderin deposition, and ductular reaction.

Alcoholic cirrhosis: in ALD, fibrosis begins in Zone 3, perivenular region and extends in a pericellular/perisinusoidal pattern. Later fibrosis progresses to extend to the portal tracts and centro-portal or porto-portal bridging fibrosis. Finally the simultaneous fibrosis and hepatocytes regeneration results in nodule formation and finally cirrhosis.8

Hepatocellular carcinoma (HCC): HCC can develop in the background of ALD cirrhosis in up to 5% -

15 %. ALD is the commonest cause of HCC in the West.[8]

B.4. Treatment of ALD

Patients with ALD are most commonly treated with the aim to eliminate alcohol intake.

Disulfiram, an irreversible inhibitor of alcohol dehydrogenase is frequently used to treat alcoholism but is not recommended for patients with advanced ALD, because of potential severe hepatotoxicity. Anticraving drugs (acamprosate), also potentially hepatotoxic, are effective at preventing relapse. Agonist of the γ-aminobutyric acid B receptor, baclofen, has been found to be effective in maintaining abstinence.

There are no approved anti fibrotic agents to prevent disease progression (6) %

B.5. Treatment of acute alcoholic hepatitis

The severe form of alcoholic hepatitis (HA) is defined by a Maddrey's discriminant function ≥ 32 or hepatic encephalopathy, and requires corticoids. Nevertheless, despite the efficacy of this treatment, mortality 6 months after the onset of disease is still 30-40%. Because some patients do not respond to existing corticosteroids, it is crucial to rapidly identify nonresponders, using scoring system such as the Glasgow Alcoholic Hepatitis Score, the ABIC (age-bilirubin-INR-creatinine) score and the Lille model. The therapeutic response is defined according three groups of patients: the complete responders, the partial responders and the non-responders. In theory, corticosteroids help in treating HA because of their anti-inflammatory properties, but severe acute AH is associated with significant lymphocyte corticosteroid insensitivity. This property can be reduced by administration of theophylline a xanthine which silences the expression of pro-inflammatory genes, or reagents that block the IL-2 receptor such as Basiliximab. Down regulation of pro-inflammatory genes in macrophages and monocytes can also be obtained by GILZ (glucocorticoid-induced leucine zipper).

For patients who cannot receive corticoids, pentoxifylline, an A xanthine, phosphodiesterase inhibitor that block transcription of TNF-α serum levels of the gene product is used.

Given the potential risk of Wernicke encephalopathy in alcoholic and malnourished patients, the administration of B-complex vitamins is often required. Patients with AH are also predisposed to develop severe infections, so early diagnosis and empiric antibiotic treatment are advised.

Some new targets to treat AH are emerged : CXC chemokine, IL22, complement, Gut micro biota and LPS pathways, inhibition of apoptosis, osteopontin, endocannabinoids and nostrin, all of these need further investigations.[6,7]

B.6. Is liver biopsy necessary in the management of alcoholic hepatitis?

Histology remains the gold standard in diagnosing acute alcoholic hepatitis (AAH); indeed, there is controversy over whether histology is essential in the diagnosis of AAH for the American Association for the study of liver disease and the European Association for the study of the liver disease who recommend to perform trans jugular liver biopsy only where there is irresolvable diagnosis uncertainty.

Indeed, characteristic changes of ASH are seen in patients with ALD without clinical syndrome of AAH or even active alcohol abuse. ASH has also been noted in explant from transplanted patients who were presumed to be abstinent. A Spanish group reported that 53% explants from ALD patients have ASH, which was not associated with a reduced survival. Therefore what is recommended is the use of ASH for histological diagnosis and the use of AAH for the clinical syndrome.

There is also little attention about the timing of the biopsy, usually not reported in clinical trials. Early biopsy may be more sensitive in confirming the diagnosis; further studies are required to establish the optimal timing of liver biopsy.

Elsewhere, histological scores to determine prognosis could be interesting. Steatosis < 20% is an independent predictor of poor outcome and polymorphonuclear cell infiltrate is associated with severity of AAH and 1 year survival. A histological severity score including K8/18 staining (a marker for hepatocytes ballooning) shows good accuracy for predicting 90% survival, but has not yet been validated. Only one validated AAH histology score has been published in abstract, finding that fibrosis stage, polymorphonuclear infiltrate, cholestasis and the presence of megamitochondria predicted 90% mortality. Further studies are required to confirm the utility of a histological score.[9,10]

In conclusion, liver biopsy remains the recommended gold standard diagnosis for ASH and has the potential to be used as a prognostic indicator.

C. NAFLD

The spectrum of disease due to hepatic fat storage is termed Non Alcoholic Fatty Liver Disease (NAFLD) which is a clinical entity related to metabolic syndrome. With the rise of obesity, NAFLD with a general prevalence of 25-30% is now considered as the most common liver disease in western countries and will be the most common etiology for liver transplantation in the 21st century. Majority of the patients are obese but the disease can also affect non-obese patients. The prevalence is increased in type 2 diabetes mellitus (70%) and morbid obesity (90%). Metabolic and genetic factors play important roles in the pathogenesis of the disease. The spectrum of disorders included in NAFLD is benign macrovesicular hepatic steatosis, nonalcoholic steatohepatitis, hepatic fibrosis, cirrhosis and hepatocellular carcinoma.[11,12]

C.1. Pathogenesis:

Metabolic syndrome and insulin resistance:

Obesity is an important risk factor for the development of NAFLD. Obesity may lead to insulin resistance and metabolic syndrome, and NAFLD is the hepatic manifestation of metabolic syndrome, but insulin resistance can also be responsible for NAFLD even in non-obese patients.

The accumulation of fat in hepatocytes can be achieved via four mechanisms: increase of free fatty acid and lipid uptake, increase in de novo lipogenesis, decreased lipid oxidation and decrease hepatic VLDL secretion via apolipoprotein B mutation.

Dietary fat in the form of chylomicron supplies free fat acid to the liver. Carbohydrate metabolism leads to de novo synthesis of free fat acid from acetylCoA. Glucose also activated promotes hepatic lipogenosis.

Hepatic steatosis is often associated with resistance to the action of insulin on hepatic gluconeogenesis, while hepatocytes and adipose tissues remain sensitive to the lipogenic action of insulin. Hepatic insulin resistance occurs when there is an excess of free fat acid influx into hepatocytes. Metabolites of FFA relocate cytoplasmic several protein kinases C to the membrane. Protein kinase C then phosphorylate intracellular portion of insulin receptors with the development of insulin resistance. In obesity, fatty acids released from adipocytes are an important source of fatty acids that are found as triacylglycerol within hepatic lipid droplets. Normally, insulin inhibits glucogenosis and promotes lipogenosis in the liver. In insulin resistant liver, glucogenosis continues leading to hyperglycemia and hyperinsulinemia while fatty acid synthesis is maintained in the liver. In the normal state, insulin also inhibits the production of VLDL.

Although initially considered as a sequential progression from simple steatosis to accompanied inflammation, it is now widely accepted that the pathogenesis of simple steatosis and NASH is likely to progress via different mechanisms. The development of NASH consists of a number of events whereby steatosis, inflammation and cell damage may occur in parallel rather than in strict sequence.

In the first hit, there is an accumulation of triglycerides as lipid droplets in the cytoplasm of more than 5% of hepatocytes. Insulin resistance contributes to this steatosis. The benign steatosis is reversible and can be self-limited but makes the liver susceptible to the second hit including oxidative stress due to the excessive fatty acid oxidation, cardiolipin peroxidation leading to mitochondrial dysfunction and more reactive oxygen species formation, pro-inflammatory cytokine formation, apoptosis and gut-derived endotoxemia. This second hit promotes NASH.

A third hit include palatine-like phospholipase domain-containing3 (PNPLA3) or adiponutrin gene involvement and impaired hepatocyte regeneration.[13]

Genetic background:

About 30% of patients with NAFLD have normal BMI. Variant single nucleotide polymorphisms in PZP and PNPLA3 genes were found to be independent risk factors for development of NAFLD. There have been found associated with higher frequency, severe histologic changes and more progression in NAFLD.[12]

The PNPLA3 I148M variant induces an increase in lipogenic activity, leading to increase hepatic triglyceride synthesis; studies in transgenic mice suggest that hepatic triglyceride level is associated with multiple changes in triglyceride metabolism. PNPLAP3I148 variant is associated in NAFLD in adults but also in children. The literature also suggests a predominant association between PNPLA3I148M variant in women but not in men. The gender differences may mainly result from variation in estrogen levels, variation in the gene or the interaction of both.

It is interesting to note that the prevalence of NAFLD differs among different populations. Hispanics have been demonstrated to have a higher prevalence of hepatic steatosis compared with European-Americans, whereas African-Americans have a lower prevalence. The frequency of the 148M variant is higher in Hispanics, indicate that PNPAL3 I148M variant may explain ethnic differences in the prevalence of NAFLD and that some of the ethnic variations are genetic. Furthermore PNPLA3 I148M variant is not associated with metabolic syndrome.

PNPLA3 I148M is also implicated in NAFLD fibrosis. It has been reported that 148M variant influences both the presence of NASH and the severity of liver fibrosis in biopsy from NAFLD patients independently of degree of obesity, diabetes and steatosis. Studies in adults but also in children reveal that this variant significantly influences the occurrence of fibrosis; however the specific mechanism is still not clear. It is speculated that I148M variant promotes the development of fibrogenesis by activating the hedgehog signaling pathway, which, in turn, leads to the activation and proliferation of hepatic stellate cells and excessive generation and deposition of extra cellular matrix.[14]

Recently, a variant of the TM6SF2 gene has been shown to be linked to fatty liver due to reduced secretion of VLDL lipoproteins.[15]

Developmental origin:

NAFLD shares a developmental origin that begins during gestation. It is recognized that pregnant women with obesity or gestational diabetes may transmit this phenotype to their offspring, leading to a vicious cycle of obesity and diabetes over generations. The proposal pathway is that the gestational environment drives increased metabolic fuel delivery to the fetus, leading to fetal fat hepatic accumulation. Via epigenic changes acquired prenatally, the live risk now programed for postnatal fat storage and inflammation mediated by Kupffer cells, a childhood high-fat diet then triggers further steatosis, hepatic injury, inflammation and fibrosis via activation of hepatic stellate cells.[11,13]

C.2. Histology

Metabolic syndrome is clinically defined by the following essential components including visceral obesity, insulin resistance, dyslipidemia, hypertriglyceridemia, decreased high density lipoprotein cholesterol, type 2 diabetes hypertension commonly found in NAFLD. Diabetes and hypertension are present up to 15-fods in patients with NASH compared to those with steatosis alone independent of age or BMI.[15]

Diagnosis of NAFLD is established if there is no significant alcohol drinking history and if there is fatty liver on imaging.

Liver biopsy remains the gold standard to estimate if the patient presents only simple steatosis or NASH, fibrosis or cirrhosis. Indeed, histological features of alcoholic and nonalcoholic liver disease are similar, so the clinical history is very important to distinguish these two entities. Classically, the NAFLD patient is a nondrinker or a social drinker but does not abuse of alcohol within the last 5 years. Moreover, the natural history of NAFLD is also largely unknown and it is not clear that all patients with steatosis are at risk for NASH or advanced cirrhosis. Patients with steatohepatitis may cycle in and out of states in which that diagnosis can be recognized histologically, depending on therapeutic intervention and/or lifestyle modifications.[16]

Histology of NAFLD: the simple steatosis is characterized by macrovesicular or mixed macro and microvesicular steatosis of more than 5% of hepatocytes. The steatosis is more prominent in the perivenular regions (zone 3). The extent of steatosis is commonly evaluated and reported semi-quantitatively. The most reproducible method follows the acinar architecture separating the liver parenchyma in third and assessing percentage involvement of steatotic hepatocytes: mild: 0-33%, moderate: 33-66% or severe : >66%.[17,18]

Histology of NASH: NASH is characterized by the triad: steatosis, ballooning degeneration and inflammation.

Ballooning degeneration, considered as the hallmark of steatohepatitis, is recognized by a swollen hepatocyte with foamy, pale cytoplasm and enlarged hyperchromatic nucleus, firstly present in zone 3. Loss of normal hepatocyte keratin 8/18 immunostaining can be helpful in the detection of the ballooned hepatocytes. Hepatocytic ballooning is the result of degeneration and fragmentation of cytoskeleton intermediate filaments, cytokeratin 8/18, aggregate of the degenerated CK8/18 is a Mallory-Denk body. Immunohistochemical staining for CK18, ubiquitin and p62 can be applied to detect hepatocellular ballooning. Typical findings show Ubiquitin positive small aggregates in CK18 negative ballooning hepatocytes.[12,19] Ballooning is a feature of major importance as its presence has been associated in prognostic studies with more aggressive disease and higher incidence of cirrhosis.[17]

Apoptotic bodies are common in NASH. The number of acidophil bodies per mm2 of liver tissue (acidophil body index) has been proposed to serve as a complementary histological feature when diagnosis of NASH in uncertain.

Mild inflammation mainly in the acini and sometimes in the portal tract is the central feature in NASH. Mixed inflammatory cells are found. Satellitosis, Mallory bodies, glycogenated nuclei, iron deposition and mega mitochondria can be rarely seen in NASH. As the disease progresses, portal inflammation becomes more severe.

Hepatic fibrosis: hepatic fibrosis generally begins in the zone 3. Fibrosis is pericellular and perisinusoidal, portal and periportal occur as well. When the disease goes on, cirrhosis occurs. At that time, the classical triad of NASH and perisinusoidal fibrosis will become less prominent or disappear so that much NASH-related cirrhosis are labeled as cryptogenic cirrhosis.

At the stage of cirrhosis, the chance to develop hepatocellular carcinoma is about 7% over 10 years. Non-cirrhotic NAFLD may also develop HCC possibly because of associated metabolic syndrome.

Histology in children shows that the lesions may or may not resemble those of adults. Schwimmer propose a schema of type 1 and 2 steatohepatitis in pediatric fatty liver. Type 1, the least common, almost in girls, resembles the adult pattern. Type 2, more common in boys, consists of either zone 1 accentuation of steatosis or panacinar steatosis. Ballooning is uncommon in both types; zone 3 perisinusoidal fibrosis is uncommon in type 2. When fibrosis is present, only portal-based fibrosis is seen.

Differential diagnosis includes ASH, drug toxicity (Tamoxifen, glucocorticoids, highly active antiretroviral therapy), metabolic diseases (Wilson disease, tyrosinemia, citrin deficiency), lipodystrophy, surgical procedures (bypass, biliopancreatic diversion), total parenteral nutrition and malnutrition.[17]

C.3. Role of liver biopsy in NAFLD

What is accepted is that steatosis itself is considered « non progressive » whereas steatohepatitis is the constellation of lesions with potential to progression. Thus the role of the biopsy could be separate individuals with NASH from those with only steatosis or steatosis and inflammation. According to the 2012 guidelines from AASLD, liver biopsy should be reserved for subjects who will benefit, for subjects with potentially competing diagnoses and for children with either an unclear diagnosis or in whom consideration is being given for medication. The EASLD recommends liver biopsy in all bariatric surgery subjects and as an endpoint in all clinical trials. A recent study reveals that performing an early liver biopsy could provide survival benefit to patients with NAFLD.

However liver biopsy is invasive, potentially harmful and may suffer from « sampling error », it is recognized as the standard evaluation for NAFLD. Liver biopsy evaluation is also important for determine an underlying cause of disease as well as autoimmune liver disease, as we know that up to 20% of NAFLD have positive serology for antinuclear, anti-smooth muscle antibodies or anti-mitochondrial antibodies. Finally, liver biopsy gives both confirmation of the diagnosis as well as evaluation and semi quantification of necroinflammatory lesions and fibrosis.[17]

Progression of fibrosis and architectural remodeling are thought to develop in 10-15% of NASH, and cirrhosis in 15-25%, so that of all individuals with some form of fatty liver, 3 -5% may develop cirrhosis.

Grading and staging: the current most widely used histological scoring system is the one published in 2005 by the NASH Clinical Research Network applicable to the entire histological spectrum of NAFLD, both in adults and pediatric NAFLD biopsies, which generates a numeric NAFLD activity score (NAS), for comparing pre- and post-treatment biopsies in therapeutic trials. NAS scores steatosis, lobular inflammation and hepatocellular ballooning, NAS ranges from 0 to 8, 1 or 2 correspond to definitely not NASH and 5 to 8 correlates with definite NASH. The fibrosis score reflects the unique pattern of fibrosis that may occur with a score range from 1 to 4.[20,21,22]

C.4. Management

The goal of management will be an early diagnosis. As most of the patients with NAFLD are overweight or obese and have associated metabolic syndrome, gradual weight loss is advocated as the first line of intervention. As NAFLD is also associated with metabolic syndrome, the associated comorbidities like diabetes mellitus, hypertension and hyperlipidemia should be managed as part of the treatment of NAFLD. The broad categories of pharmacotherapy for the treatment of NAFLD include: antioxidants as vitamin E associated with improvement in steatosis and steatohepatitis, Insulin-sensitizing agents like Thioglitazones, agonist of peroxisome proliferator-activated receptor gamma that control transcription of insulin receptor genes involved in the transport, utilization and production of glucose and lipids, miscellaneous agents like Pentoxifyllin, a xanthine derivative associated in improving steatosis and lobular inflammation and in one study ballooning degeneration. Research is ongoing to find out prevention and better therapeutic options, Sirtuins that are silent information regulator proteins which act as nicotinamide adenine dinucleotide dependent deacylases and can thus modulate activation and deactivation of certain proteins are of interest. SRT1 can be a potential target in the treatment of NAFLD.[12]

D. Cholesterol ester storage disease (CESD)

Lysosomal acid lipase (LAL) deficiency is a rare autosomal recessive progressive metabolic genetic liver disease, characterized by low level or absence of activity of the LAL enzyme leading to the lysosomal accumulation of lipids predominantly in macrophages but also seen in hepatocytes. CESD can easily be confused with NAFLD and is underdiagnosed. A careful research of microvesicular steatosis which predominate with minimal zonal differences within the hepatic lobule, and macrophages that have accumulated both lipids and ceroid, are helpful to suspect the diagnosis. The histological diagnosis of CESD is facilitated by immunostaining for the lysosomal protein, cathepsin D, which is routinely performed in many laboratories. The diagnosis is confirmed by demonstrating the markedly deficient LAL activity or by mutation analysis.[23,24]

References

1. Tomohiro S, Satako S et al. Hypoxia and fatty liver.World J Gastroenterology 2014 Jun 21; 20(41): 15087-15097.
2. Burt AD, Portman BC, Ferrell LD. Mac Sween's Pathology of the liver. Churchill Livingstone Elsevier 2007.
3. Greenberg AS, Coleman RA et al. The role of lipid droplets in metabolic disease in rodents and humans. J Clin Invest 2011; 121(6): 2102-2110.
4. Krahmer N, Farese R et al. Balancing the fat: lipid droplets and human disease. EMBO Mol Med 2013; 5: 905-915.
5. Rocco A, Copare D et al. Alcoholic disease: liver and beyond. World J Gastroenterology 2014 October 28; 20(40): 14652-14659.
6. Gao B and Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology 2011; Nov 141(5): 1572-1585.
7. Louvets A and Mathurin P. Alcoholic liver disease: mechanisms of injury and targeted treatment. Nature review Gastroenterology & Hepatology 2015; 12:231-242.
8. Levy RE, Catana AM et al. Ethnic differences in presentation and severity of alcoholic liver disease. Alcohol Clin Exp Res 2015; Mar; 39(3): 566-74.
9. Sakhuja puia. Pathology of alcoholic liver disease, can it be differentiated from nonalcoholic steatohepatitis? World J Gastroenterology 2014 Nov 28; 20(44): 16474-16479.
10. Dhanda AD, Collins PL and McCune CA. Is liver biopsy necessary in the management of alcoholic hepatitis? World J Gastroenterology 2013 November28; 19(44): 7825-7829.

INVITED LECTURES

11. Brumbaugh DE and Friedman JE. Developmental origins of nonalcoholic fatty liver disease. Pediatr Res. 2014 Jannuary; 75(0): 140-147.
12. Monjur Ahmed. Non-alcoholic fatty liver disease in 2015. World J Hepatol 2015 June 18; 7(11): 1450-1459.
13. Li M, Reynolds CM et al. Developmental Programming of Nonalcoholic Fatty Liver Disease: The Effect of Early Life Nutrition on Susceptibility and Disease Severity in Later Life. BioMed research International 2015; Article ID 437107, 12 pages.
14. Chen L-Z, Xin Y-N et al. PNPLA3I148M variant in nonalcoholic fatty liver disease: demographic and ethnic characteristics and the role of the variant in nonalcoholic fatty liver fibrosis. World J Gastroenterology 2015 Jan 21; 21(3): 794-802.
15. Azzam H and Malnick S. Non-alcoholic fatty liver disease- the heart of the matter. World J Hepatol 2015 June8; 7(10): 1369-1376.
16. Kleiner DE and Brunt EM. Nonalcoholic Fatty Liver Disease: Pathologic Patterns and Biopsy Evaluation in Clinical Research. Semin Liver 2012; 32: 3-13.
17. Brunt EM and Tiniakos DG. Histopathology of nonalcoholic fatty liver disease. World J Gastroenterology 2010 November 14; 16(42): 5286-5296.
18. Nalbantoglu I and Brunt EM. Role of liver biopsy in nonalcoholic fatty liver disease. World J Gastroenterology 2014 July 21; 20(27): 9026-9037.
19. Ikura Yoshihiro. Transitions of histopathologic criteria for diagnosis of nonalcoholic fatty liver disease during the last three decades. World J Hepathology 2014 Dec 27; 6(12): 894-900.
20. Brunt EM, Janney CG et al. Nonalcoholic Steatohepatitis: a Proposal for Grading and Staging the Histological Lesions. Am J Gastoenterol 1999: 2467-2474.
21. Kleiner DE, Brunt EM et al. Design and Validation of a Histological Scoring System for Nonalcoholic Fatty Liver Disease. Hepatology 2005; 41: 1313-1321.
22. Brunt EM, Kleiner DE et al. The NAS and the Histopathologic Diagnosis in NAFLD: Distinct Clinicopathologic Meanings. Hepatology 2011 March; 53(3): 810-820.
23. Hůlkova H and Elleder M. Distinctive histopathological features that support a diagnosis of cholesterol ester storage disease in the liver biopsy specimens. Histopathology 2012; 60: 1107-1113.
24. Bernstein DL, Hůlkova H et al. Cholesteryl ester storage disease: review of the findings in 135 reported patients with underdiagnosed disease. J Hepathology 2013; 58: 1230-1243.

L 25

CT AND MRI OF DIFFUSE LIVER DISEASE.

Dymarkowski S.

CT and MRI contribute important information to the clinical evaluation of diffuse liver disease. Characteristic alterations of liver attenuation on CT, signal changes on MRI, and morphological changes appreciated with both modalities can be used to diagnose fatty infiltration, some parenchymal deposition diseases, and cirrhosis. Furthermore, hepatocellular disease can be confirmed in the setting of indeterminate clinical and laboratory findings. The prominent role of multidetector CT is primarily defined by its excellent morphologic visualization capabilities, in particular of diffuse or focal intrahepatic lesions as well as of anatomic relationships between the liver and adjacent organs. Significant overlap in the imaging findings of this wide range of disorders continues to limit specificity; however, at a minimum, these techniques provide a rapid means to a noninvasive evaluation that often guides clinical decisions. MR imaging has been proved to be a comprehensive modality for assessing the morphology and functional characteristics of the liver. Concurrent technical improvements as well as implementation of advanced imaging sequence designs permit high-quality examination of the liver with T1-, T2-, and diffusion-weighted pulse sequences.

Three basic demands remain if MR imaging is chosen for hepatic imaging: to improve parenchymal contrast, to suppress respiratory motion, and to ensure complete anatomic coverage. Faster scanning techniques available with CT and MRI may provide additional information by assessing contrast dynamics. This review of CT and MRI in diffuse liver disease considers the diagnostic utility and clinical implications of these modalities.

INVITED LECTURES

L 26

CLINICOPATHOLOGICAL ASPECTS OF DRUG-INDUCED HEPATITIS.

P. Starkel and M.Komuta

Drug-induced hepatitis may present all kinds of pathological features, such as acute, chronic, vascular, cholestatic, and neoplastic hepatic diseases. This makes us difficult to understand and reach a correct diagnosis.

In this session, we are going to clarify the clinicopathological aspects of different kinds of drug-induced hepatitis based on their pathological aspects (patterns): Clinical aspects will be first presented then after pathological aspects will be reviewed.

We are hoping that the session, clinicopathological correlation in drug-induced hepatitis may help for your daily practise.

L 27

PITFALLS IN DIAGNOSIS OF NEUROENDOCRINE TUMORS OF THE LUNG

William D. Travis, M.D. Attending Thoracic Pathologist Memorial Sloan Kettering Cancer Center New York, NY

PITFALLS IN LUNG NET

- Morphologic pitfalls
- Immunohistochemical pitfalls
- Genetic pitfalls
- Conclusions

MORPHOLOGIC PITFALLS

- Small Biopsies/Cytology
- Poor preservation of tumor
    - Frozen section
    - Poor quality H&E
- Carcinoid mimickers
- SCLC mimickers
- LCNEC mimickers

IMMUNOHISTOCHEMICAL PITFALLS

- NE markers in non-NE tumors
- Lack of NE markers in NE tumors
- TTF-1 expression in
    - Carcinoid tumors
    - SCLC/LCNEC
- Ki-67 staining
    - Main role – separating carcinoids from high grade SCLC or LCNEC
    - Not reliable in distinguishing TC from AC

GENETIC PITFALLS

- EGFR mutations in SCLC/LCNEC
- Think of combined adenocarcinoma if patient never smoker
- If adenocarcinoma component present in combined SCLC or LCNEC, tumor should be sent for molecular testing

PITFALLS CONCLUSIONS

- Start with good quality H&E stain and history (age, smoking hx, number & location of lesions)
- If morphology, stains and clinical picture don't fit, show to colleague and think carefully about "pitfall" differentials

L 28

GASTRIC ECL-CELL TUMOURS AND RELATED PRECURSOR LESIONS

Stefano La Rosa, MD - Department of Pathology, Ospedale di Circolo - Viale Borri 57, 21100 Varese, Italy - stefano.larosa@ospedale.varese.it

Although up to five neuroendocrine cell types have been characterized in the human gastric mucosa [1], most gastric neuroendocrine tumours (NETs) are composed of histamine-producing ECL-cells [2,3]. Gastric ECL-cell NETs account for about 4% of all neuroendocrine neoplasms of the body and 66% of all gastric neuroendocrine neoplasms. Their annual incidence has been increased in the last 40 years and accounts for 0.30 per 100,000 population with a female prevalence and a mean age at diagnosis of 64 years. Patients' survival progressively improved during the same period with a 5-year survival of 64.1% [4].They represent a heterogeneous group of neuroendocrine proliferations showing different clinicopathologic features and behaviour [2,5,6] and are currently classified, using the 2010 WHO classification of digestive neuroendocrine neoplasms, into two different categories: i) grade 1 NETs (G1 NETs) showing < 2 mitoses x 10HPF and < 3% Ki67 proliferative index; ii) G2-NETs showing 2-20 mitoses x 10HPF and/or 3-20% Ki67 index [7].

Hyperplastic and dysplastic proliferations of ECL-cells have been traditionally considered as precursors of ECL-cell NETs [8-10], but it has recently been demonstrated that their show different neoplastic potential ranging from lesions lacking any risk of tumour development (i.e. diffuse ECL-cell hyperplasia) to dysplastic lesions with a statistical increased risk for tumour development [10].

Specific types of ECL-cell NETs

Although ECL-cell NETs can be classified as either G1- or G2-NETs depending on the proliferative activity as above mentioned [2], the clinicopathologic background of patients has a great prognostic impact [2,5,11] and needs to be considered when diagnosing a gastric ECL-cell NET. Consequently, the integration of the 2010 WHO classification together with the clinicopathologic context improves the prognostic evaluation. Considering the morphology of peritumoural oxyntic mucosa, the presence of antral G-cells hyperplasia, the presence of hypergastrinemia, the setting of MEN1, and the presence of hypo/achlorhydria, ECL-cell NETs can be divided into the following four categories: Type 1, Type 2, Type 3, and Type 4 [11-13].

Type 1 ECL-cell NET

Type 1 ECL-cell NETs arise in the setting of autoimmune chronic atrophic gastritis (type A CAG) in patients showing hypergastrinemia due to a compensatory hyperplasia of antral G-cells in response to hypo/achlorhydria depending on the loss of exocrine glands. Most tumours are less than 1.5 cm in size and are composed of well differentiated cells growing in trabecular structures generally confined to mucosa or submucosa [2,14]. They show very low proliferation, being classified as G1-NETs in most of cases [2]. Tumour cells are positive for synaptophysin, chromogranin A, vesicular monoamine transporter 2 (VMAT2), and somatostatin receptor 2A [2,15].

The peritumoural oxyntic mucosa is atrophic and shows hyperplastic and/or dysplastic proliferations of ECL-cells. Type 1 NETs are relatively indolent tumours that are rarely metastatic to lymph nodes with a reported tumour-associated death of 1.07% [2].

Type 2 ECL-cell NET

Type 2 NETs account for about 8% of gastric ECL-cell NETs [2,14]. Like Type 1 NETs they arise in the oxyntic mucosa in patients with hypergastrinemia, which depends on the presence of a duodenal or, more rarely, a pancreatic gastrinoma in the setting of MEN1 syndrome. In contrast to Type 1 NETs, peritumoural mucosa is hypertrophic. Tumours are usually multiple and less than 2 cm in size. Tumours are composed of well differentiated cells growing in trabecular structures confined to mucosa-submucosa in 90% of cases and the immunohistochemical profile overlaps that of Type 1 NETs. Most tumours are G1-NETs. However, lymph node and distant metastases can be detected in 30% and 10% of patients, respectively and the prognosis is worse than that of Type 1 NETs.

In peritumoural hyperplastic oxyntic mucosa, various types of hyperplastic/dysplastic ECL-cell proliferations can be found [4].

Type 3 ECL-cell NET

Type 3 NETs arise more frequently in males with normal oxyntic mucosa lacking hyperplastic and/or dysplastic ECL-cell proliferations. They represent about 23% of all gastric ECL-cell NETs and the mean age of patients at diagnosis is about 50 years. Tumours are single and large with a diameter around 2 cm. In more than 70% of cases, Type 3 NETs infiltrate the muscular layer with lymph node metastases. Liver metastases are present in about 50% of patients [2,14]. Histologically, some tumours resemble Type 1 or 2 NETs and are composed of monomorphic cells with regular nuclei growing in trabecular/gyriform structures. Others tumours show large and disordered trabeculae composed of larger cells with crowded nuclei showing moderate atypia, less regularly distributed chromatin and mitoses [2].

Type 3 NETs can show different proliferative degrees ranging from cases classified as G1- or G2-NETs to rare cases falling in the G3 category. Both G1 and G2-NETs can develop lymph node metastases, so the biology of Type 3 NETs does not strictly depend on proliferative indices. The group of Type 3 NETs associated with high proliferation (G3) is a challenging task for pathologist with relevant clinical implications, since, according to the 2010 WHO criteria, they should be classified as NECs and consequently treated with platinum-based chemotherapy but, as recently demonstrated, this therapeutic approach is not the gold standard for tumours with these characteristics [16,17].

Type 4 ECL-cell NETs

Type 4 NET is a recently described tumour type arising in association with hypergastrinemia and parietal cell hypertrophy not associated with MEN1-ZES [12,13]. Tumours are multiple and the peritumoural mucosa shows hyperplastic oxyntic glands and different types of ECL-cell proliferations. The morphological features of tumours overlap those of Type 1 and 2 NETs. Lymph node metastases have been found in one of the two reported cases [13]. The mechanism underlying hypergastrinemia in absence of both autoimmune gastritis and gastrinoma seems to be related to a defect in acid secretion from parietal cells due to a defect/lack of proton pump.

Tumour staging

The histology report must also include tumour stage which has been demonstrated to predict prognosis [18]. A TNM staging system for gastric NENs was first proposed by ENETS in 2006 [19]. Successively, the International Union for Cancer Control and the American Joint Cancer Committee (UICC/AJCC) developed another TNM classification [20]. These two staging systems are different and no studies have been conducted to compare them to show which one is better in order to stratify patients in different prognostic categories. Interestingly, the number of metastatic lymph nodes (proposed N1: < 3 lymph nodes; N2: > 3 lymph nodes), not considered in both schemes, has recently been demonstrated to have an important prognostic significance [2]. Further studies are needed to clarify which is the better staging system to use.

Hyperplastic and dysplastic ECL-cell proliferations

ECL-cell proliferations have been traditionally considered precursors of ECL-cell NET development and include a wide range of different growths: i) hyperplastic lesions: diffuse, linear, micronodular, and adenomatoid hyperplasia; ii) dysplastic lesions: enlarged micronodules, fused micronodules, micronodules with new stroma, and microinfiltrative lesions [8].

Among the different ECL-cell lesions, only severe ECL-cell hyperplasia, characterized by more than 6 chains of linear hyperplasia per mm, and ECL-cell dysplasia, mainly represented by microinfiltrative lesions, showed a significant increased risk for Type 1 NET development [10]. This finding may suggest the need for endoscopic and histologic surveillance only to the patients with ECL-cell dysplasia/severe hyperplasia.

In contrast to Type 1 NETs, in Type 2 NETs linear hyperplasia alone is by itself highly predictive of NET development [21]. Interestingly, although hyperplastic ECL-cell lesions can also be observed in the oxyntic mucosa of patients with gastrinoma without MEN1 syndrome, both ECL-cell dysplasia and ECL-cell NETs only develop in patients with MEN1 syndrome suggesting that the genetic changes in MEN1 patients render ECL-cells more sensitive to the proliferative effect of gastrin [21,22].

In patients with Type 3 NETs no hyperplastic and/or dysplastic ECL-cell proliferations are found in the peritumoural oxyntic mucosa.

Conclusions

Gastric ECL-cell NETs are a heterogeneous group of tumours showing different clinicopathologic features and behaviour. They can be classified into G1- and G2-NETs but the integration of the 2010 WHO classification [7] with the clinicopathologic classification proposed by Rindi and coworkers in 1993 [11] greatly improves the prognostic stratification of patients [2]. For this reason both approaches should be used and integrated when first diagnosing a gastric ECL-cell NET in small biopsy specimens. Indeed, G1-NETs have a good prognosis and do not need further treatments when they are small, confined to mucosa and arising in the setting of type A CAG. Tumours with similar morphological features and proliferative indices, but arising in MEN1-ZES patients or, especially, arising sporadically in normal gastric mucosa show a more aggressive biology needing further treatments including gastric resection. For this reason the morphological evaluation of both tumour and peritumoural mucosa represents the best approach to give the clinician all the information needed to choose the best treatment. Also for this reason, biopsies of peritumoural gastric mucosa must be requested when they are omitted at the first endoscopic examination.

References

1. Solcia E, Capella C, Vassallo G, et al. Endocrine cells of the gastric mucosa. Int Rev Cytol 1975;42:223-286
2. La Rosa S, Inzani F, Vanoli A, et al. Histologic characterization and improved prognostic evaluation of 209 gastric neuroendocrine neoplasms. Hum Pathol 2011;42:1373-1384.

3. Solcia E, Arnold R, Capella C, et al. Neuroendocrine neoplasms of the stomach. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. WHO classification of tumours of the digestive system. Lyon: IARC Press 2010:64-68.
4. La Rosa S, Vanoli A. Gastric neuroendocrine neoplasms and related precursors lesions. J Clin Pathol 2014;67:938-948.
5. Rindi G, Azzoni C, La Rosa S, et al. ECL cell tumor and poorly differentiated endocrine carcinoma of the stomach: prognostic evaluation by pathological analysis. Gastroenterology 1999;116:532-542.
6. Modlin IM, Kidd M, Latich I, et al. Current status of gastrointestinal carcinoids. Gastroenterology 2005;128:1717-1751.
7. Rindi G, Arnold R, Bosman FT, et al. Nomenclature and classification of neuroendocrine neoplasms of the digestive system. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. WHO classification of tumours of the digestive system. Lyon: IARC Press 2010:13-14.
8. Solcia E, Bordi C, Creutzfeldt W, et al. Histopathological classification of nonantral gastric endocrine growths in man. Digestion 1988;41:185-200.
9. Annibale B, Azzoni C, Corleto VD, et al. Atrophic body gastritis patients with enterochromaffin-like cell dysplasia are at increased risk for the development of type I gastric carcinoid. Eur J Gastroenterol Hepatol 2001;13:1449-1456.
10. Vanoli A, La Rosa S, Luinetti O, et al. Histologic changes in type A chronic atrophic gastritis indicating increased risk of neuroendocrine tumor development: the predictive role of dysplastic and severely hyperplastic enterochromaffin-like cell lesions. Hum Pathol 2013;44:1827-1837.
11. Rindi G, Luinetti O, Cornaggia M, et al. Three subtypes of gastric argyrophil carcinoid and the gastric neuroendocrine carcinoma: a clinicopathologic study. Gastroenterology 1993;104:994-1006.
12. Ooi A, Ota M, Katsuda S, et al. An unusual case of multiple gastric carcinoids associated with diffuse endocrine cell hyperplasia and parietal cell hypertrophy. Endocr Pathol 1995;6:229-237.
13. Abraham SC, Carney JA, Ooi A, et al. Achlorhydria, parietal cell hyperplasia, and multiple gastric carcinoids. A new disorder. Am J Surg Pathol 2005;29:969-975.
14. Modlin IM, Lye KD, Kidd M. Carcinoid tumors of the stomach. Surg Oncol 2003;12:153-172.
15. Rindi G, Paolotti D, Fiocca R, et al. Vesicular monoamine transporter 2 as a marker of gastric enterochromaffin-like cell tumors. Virchows Arch 2000;436:217-223.

16. Sorbye H, Welin S, Langer SW, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. Ann Oncol 2013;24:152-160.
17. Vélayoudom-Céphise FL, Duvillard P, Foucan L, et al. Are G3 ENETS neuroendocrine neoplasms heterogeneous? Endocr Relat Cancer 2013;20:649-657.
18. Rindi G, Bordi C, La Rosa S, et al. Gastroenteropancreatic (neuro) endocrine neoplasms: the histology report. Dig Liver Dis 2011;43S:S356-S360.
19. Rindi G, Klöppel G, Alhman H, et al. TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. Virchows Arch 2006;449:393-401.
20. Compton CC, Byrd DR, Garcia-Aguilar J, et al. AJCC cancer staging atlas. 2nd edition. New York: Springer 2012
21. Berna MJ, Annibale B, Marignani M, et al. A prospective study of gastric carcinoids and enterochromaffin-like cell changes in multiple endocrine neoplasia type 1 and Zollinger-Ellison syndrome: identification of risk factors. J Clin Endocrinol Metab 2008;93:1582-1591.
22. Peghini PL, Annibale B, Azzoni C, et al. Effect of chronic hypergastrinemia on human enterochromaffin-like cells: insights from patients with sporadic gastrinomas. Gastroenterology 2002;123:68-85.

L 29

INTESTINAL NET, NEC AND MANEC

Aurel Perren, Anja Schmitt - Institute of Pathology - University Bern Switzerland

Neuroendocrine neoplasias (NEN) are divided into well-differentiated neuroendocrine tumors (NET) and poorly differentiated neuroendocrine carcinomas (NEC). NEC (and rarely NET) can be admixed with a non-endocrine component. If this component encompasses between 30% and 70% of the neoplasm, the definition of a Mixed Adeno-Neuro-Endocrine Carcinoma (MANEC) is fulfilled.

From a practical viewpoint, ileal NET do not pose a diagnostic problem. If the tumor manifests clinically as liver metastasis without detectable primary tumor (CUP), the examination of the three transcription factors Ttf-1 (lung, thyroid), Islet-1 (pancreas) and cdx-2 (ileum, intestinal tract) can be of help in addition to the use of a hormonal profile [1]. These stainings cannot be used in the setting of NEC. Up to 30% of small intestinal carcinoids are multifocal, a possible hint to familiarity. Recently, a 4bP deletion in the IPMK gene was reported in one such family, however other families did not show this mutation, indicating familial small intestinal NET might be a heterogeneous disease [2].

NET of the Appendix are clinically almost always benign if smaller than 1cm. Tumors >2 cm bear a relevant risk of metastasis. The ENETS staging proposal differs significantly from the UICC staging system of appendiceal NET and therefore all the histopathological "facts" need to be reported [3]. Goblet cell carcinoids lead to a confusion due to the name of the disease and are probably best treated and staged as low grade adenocarcinomas.

A large fraction of colonic neuro-endocrine neoplasia is represented by poorly differentiated NEC. Even in their pure form, they seem to represent high-grade transformations of adenocarcinomas, as they frequently arise in association with adenocarcinomas. If mixed with adenocarcinoma, genetic analysis has proven the monoclonal origin [4]. The cut-off for the diagnostic criterion of a mixed endocrine non-endocrine carcinoma is artificial and the clinical implication of the category of MANEC is unsolved. Rectal NET differ from colonic NEN, they are most frequently incidental findings and, if smaller than 1cm and without invasion of the muscularis mucosae cured by endoscopic resection. Rectal NET are often chromogranin-A negative and care must be taken in the differential diagnosis to adenocarcinomas in small crushed biopsies.

1. Schmitt, A.M., et al., Islet 1 (Isl1) expression is a reliable marker for pancreatic endocrine tumors and their metastases. Am J Surg Pathol, 2008. 32(3): p. 420-5.
2. Sei, Y., et al., A Hereditary Form of Small Intestinal Carcinoid Associated With a Germline Mutation in Inositol Polyphosphate Multikinase. Gastroenterology, 2015.
3. Pape, U.F., et al., ENETS Consensus Guidelines for the management of patients with neuroendocrine neoplasms from the jejuno-ileum and the appendix including goblet cell carcinomas. Neuroendocrinology, 2012. 95(2): p. 135-56.
4. Scardoni, M., et al., Mixed adenoneuroendocrine carcinomas of the gastrointestinal tract: targeted next-generation sequencing suggests a monoclonal origin of the two components. Neuroendocrinology, 2014. 100(4): p. 310-6.

INVITED LECTURES

L 31

THE DNET REGISTRY : A PROSPECTIVE, NATIONAL, WEB-BASED ONLINE REGISTRY OF DIGESTIVE NEURO-ENDOCRINE TUMOURS. STATUS AFTER 30 MONTHS OF INCLUSION.

I. Borbath, on behalf of the Belgian Group of Digestive Oncology.

Introduction:

Digestive Neuro-Endocrine tumours (NET) are rare and poorly understood neoplasms that deserve a better understanding. It was therefore the project of the BGDO to create a web-based registry to have an overview of incidence, diagnostic and therapeutic procedures performed in Belgian patients. A new version of the DNET was launched in 01/2012. Patients diagnosed with a NET after 01/01/2000 were included. We report data after 30 months of inclusion.

Material and methods:

As of 30 september 2015, 726 patients and more than 2957 visits were included in the registry, from 31/41 active sites. The sites had first to make the study approved by their ethics committee, all the patients had to sign informed consent. The registry is available upon username/password on www.bgdo.org/DNET.

Results:

Patients consist of 391 men and 335 female. Mean age is 62+/- 18 years. ECOG Performance Status was 0 in 398 pts (55,7%), 1 in 181 (25,3%), 2 in 28 (3,9%), 3 in 6 (0,8%), 4 in 2 (0,3%) and not known in 99 (13,8%) - information missing for 15 patients. Primary tumours are mainly pancreas (n=225, 32,9 %), midgut - including appendix - (n=224, 32,7%); information missing for 46 patients. Chromogranin A was analysed in 366, and pathologic in 235 (64%) patients. Functional syndrome (236 missing information) was present in 94/483 pts (19,5%). Octreoscan was positive in 103/141 pts (Se 73%). CytologyHistology material was available in 173 (77%) pNET; biopsy or surgical specimen was available for 158/174 midgut pts (91%). Among operated Midgut pts where ki67 was available (n=104), they were mainly WHO 2010 NET G1 (62/104, 59,6%) or NET G2 (33/104, 32%) than NEC G3 (6/104, 5,7%). Among operated pNET where ki67 was available (n=87), pts were more NET G1 (25/87, 36%), NET G2 (48/87, 55%) than NEC G3 (14/87, 16%). Surgery (403 answers as of 30 Aug 2015) was performed in a curative intent in 324 pts, for palliative reasons in 40 pts, not done in 24 pts. At the time of initial diagnosis, "watch and wait" policy was applied to 32 pts (16/32 pNET), biotherapy (somatostatin analogues and IFN) were given to 179/726 pts. During follow-up, 51 pts died (31M/20F), mainly from tumour progression (38/51 pts)

Conclusion:

The new version of the DNET registry is recruiting very well, considering the high inclusion rate (20 pts/months!) and is an effective tool for getting important informations on DNET therapy and follow-up in Belgium. Available information lacks exhaustivity, but data quality/quantity is improving over time.

L 32

THE SPECTRUM OF NEUROENDOCRINE TUMORS OF THE LUNG IN VIEW OF THE NEW WHO CLASSIFICATION

6th Belgian Week of Pathology
Ghent, Belgium
Friday, October 23, 2015

William D. Travis, M.D.
Attending Thoracic Pathologist
Memorial Sloan Kettering Cancer Center
New York, NY

NE LUNG LESIONS

- **NE cell hyperplasia**
  - **Diffuse idiopathic pulmonary NE cell hyperplasia (DIPNECH) – a rare preinvasive lesion for carcinoid tumors**
- **Tumorlet (<0.5 cm)**
- **NE Tumors**
  - **Typical carcinoid**
  - **Atypical carcinoid**
  - **Large cell neuroendocrine carcinoma**
  - **Small cell carcinoma**

2015 WHO CLASSIFICATION NEUROENDOCRINE TUMORS

- **Small cell carcinoma**
  - **Combined SCLC**
- **Large cell neuroendocrine carcinoma**
  - **Combined LCNEC**

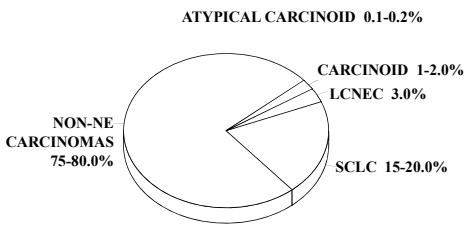
- **Carcinoid tumor**
  - **Typical carcinoid**
  - **Atypical carcinoid**

Pulmonary neuroendocrine (carcinoid) tumors: European Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids

M. E. Caplin[1*], E. Baudin[2], P. Ferolla[3], P. Filosso[4], M. Garcia-Yuste[5], E. Lim[6], K. Oberg[7], G. Pelosi[8], A. Perren[9], R. E. Rossi[1,10] & W. D. Travis[11] the ENETS consensus conference participants[†]

Caplin ME et al: Ann Oncol 2015;26:1604-20

LUNG NE TUMOR FREQUENCY



2015 WHO CLASSIFICATION SMALL CELL CARCINOMA

Variant
Combined small cell carcinoma

i.e. Combined small cell carcinoma with adenocarcinoma, squamous carcinoma, giant cell and/or spindle cell carcinoma

SCLC DIAGNOSIS AND SPECIAL STAINS

- SCLC diagnosis is made reliably in small biopsies – most can be diagnosed by H&E
- Cytology is a powerful diagnostic tool for SCLC – often is better than biopsy
- The most important stain for SCLC diagnosis is a good quality H&E
- IHC panel (if needed): AE1/AE3, TTF-1, CGA, SYN, CD56, Ki-67

LARGE CELL NE CARCINOMA DIAGNOSTIC CRITERIA

- NE Morphology: Organoid nesting, trabecular, palisading, rosette-like patterns
- Increased Mitoses (11 or more per 10 HPF or 2mm^2; Avg. 60)
- FEATURES OF A NON-SMALL CELL CARCINOMA
  - Large cell size (> diameter 3 lymphocytes)
  - Low N/C ratio (abundant cytoplasm)
  - Round to oval or polygonal shape
  - Nucleoli frequent and prominent (not every case)
  - Chromatin usually coarse or vesicular, may be finely granular
- NE Differentiation by EM or Immunohistochemistry

TYPICAL AND ATYPICAL CARCINOID DIAGNOSTIC CRITERIA

- TYPICAL CARCINOID
  - Less than 2 mitoses per 10 HPF (2 mm^2) and No foci of necrosis
- ATYPICAL CARCINOID
  - 2-10 mitoses per 10 HPF (2 mm^2) OR
  - Foci of necrosis
- Pleomorphism, cellularity, and vascular invasion are more subjective

Travis WD, et al; Am J Surg Pathol 22:934-44, 1998; 2015 WHO Classification

TNM FOR LUNG CARCINOIDS

- TNM is a useful predictor of survival
- N and M factors are strong predictors of survival
- T factor details are limited in both IASLC and SEER databases
- T factors that need more detailed evaluation:
  - Size
  - Multiple nodules (ipsilateral same/separate lobe vs contralateral)
  - < 2 cm distal to carina
  - Atelectasis
  - Pleural invasion
- Cannot assess typical vs atypical carcinoid in these datasets

Travis WD et al: JTO 3:1213-1223, 2008

PREINVASIVE NE LESIONS

PREINVASIVE LESION	NE TUMOR
▪ DIPNECH	▪ Carcinoid tumors • Typical carcinoid • Atypical carcinoid
▪ No Recognized Preinvasive Lesion	▪ LCNEC ▪ SCLC

NE LUNG TUMORS PATHOLOGIC DIFFERENTIAL

	TC	AC	LCNEC	SCLC
Mitoses per 2mm^2	<2	2-10	>11 (median-70)	>11 (median-80)
Necrosis	No	Yes	Yes	Yes
Histologic heterogeneity	No	No	Yes	Yes
MEN I Mutation Syndrome	No Yes	Yes Yes	Rare No	No No

L 36

HPV and cervical cancer

John-Paul Bogers, MD, PhD, MPH
Ina Benoy, Apr, PhD
Davy Vanden Broeck, MSc, PhD

Applied Molecular Biology Research Group (AMBIOR)
University of Antwerp
Algemeen Medisch Laboratorium (AML) – Sonic HealthCare
Antwerp – Belgium

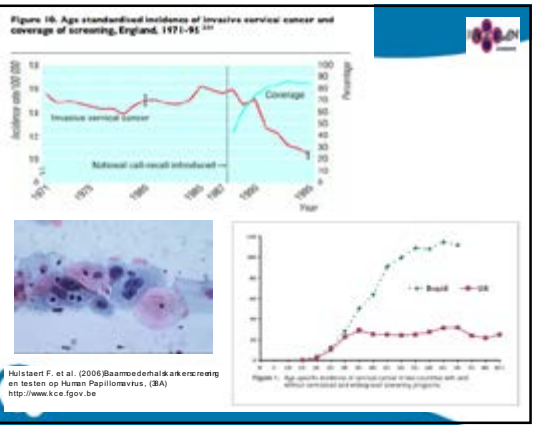
jpbogers@ambior.org

Human Papillomavirus

- Extremely frequent in the population
  - HPV infections are very common in sexually active people
  - HPV infections peak in the age group 18-25
  - More than 100 different genotypes are described
- Cervical cancer is caused by HPV infection
  - Risk is correlated with HPV type and duration of infection
    - Currently no validated markers to determine if a HPV infection is recent (transient) or persistent
  - Why all cervical cancer and not all penile/H&N/anal/esophageal?

Cytological screening

- Value is proven!
- Limited sensitivity per screening episode, good specificity
  - Good positive predictive value
  - Low negative predictive value, needs regular repeating
- Sensitivity dependent on pre-analytical phase
  - Quality improved since liquid based cytology
- Cheap, labor intensive, needs rigorous quality assessment



Hulstaert F. et al. (2006)Baarmoederhalskankerscreening en testen op Human Papillomavirus, (3BA) http://www.kce.fgov.be

Human Papillomavirus in screening

- HPV alone
- In combination with cytology
  - Cytology followed by HPV testing - Reflex HPV
    - Current guideline in most European countries and USA for equivocal smears
    - Does not solve problems of sensitivity
  - HPV followed by cytology – Reflex cytology
  - HPV and cytology together - Co-testing

HPV testing

- Complicated by very low cancer/infection ratio
  - Most (young) women can/will be positive at a specific time without relevant disease/increased risk
  - Most infections will resolve spontaneously
  - Counseling implications/ can cause stress
    - Paramount importance of clinician engaging in individual counseling, explaining the test and the relevance to each woman
    - HPV testing is perceived as a test for a sexually transmitted disease and not as risk stratification for cancer
    - Stress was not proven to be a cause of psychosocial distress (ARTISTIC)
      - Reality in Belgium often perceived differently

Kitchener, H.C. (2009) Health technol assess, 13(51), pp. 1-150, iii-iv

INVITED LECTURES

HPV testing

- Positive HPV test causes "stress"
  - On the individual
    - Absolute risk for cancer often over estimated
    - Sexually transmitted disease
    - Victim-blaming (role of behavior & life style issue), may impact attitude towards screening
  - On the healthcare system
    - Follow-up of women
      - At risk therefore ethical pressure of follow-up and treatment
      - Price of follow-up (colposcopy, gynecology service, lab,...)
    - Morbidity of treatment
      - Often overtreatment
      - Obstetric risks

HPV testing

- Combined HPV-cytology (co-testing or reflex)
  - Increase PPV
  - High sensitivity and high NPV
  - Diminish overtreatment
  - Need for trained cytotechnologists!!
    - Extremely difficult slides
    - Increase in screening time/slide
- Primary HPV screening
  - Problem with low PPV – high FPR
  - High NPV & high Sensitivity

HPV negative real lesions!

- Kaiser permanente study
  - 331,818 women
  - 5-year cumulative incidence
  - Conventional cytology with FocalPoint screening
  - Hybrid Capture 2 testing for HPV
  - "the cumulative incidence of cancer
    - 3.8/100000 women/year (HPV-)
    - 3.2/100000 women/year (HPV- and cyto -)
    - 7.5/100000 women/year (cyto -)"
- BUT...

Katki H.A., Kinney W.K. et al (2011) *Lancet oncology*, 12(7), pp. 663-72

Kaiser permanente study

- First round
  - 22% of CIN2
  - 16% of CIN3
  - 20% of AIS
  - 37% of SCC
  - 22% of adenocarcinoma
  - Thus 20% of relevant disease
- Was HPV negative.....

Katki H.A., Kinney W.K. et al (2011) *Lancet oncology*, 12(7), pp. 663-72

HPV negative real lesions!

- POBASCAM trial
  - 44,938 women
  - HPV versus cytology
    - Conventional cytology
    - GP5+/GP6+ DNA testing
  - No significant difference in the detection of CIN3+ in baseline group
    - More cases of CIN2+ in HPV group
  - The cumulative detection of CIN3+ (and of CIN2+) did not differ between the two arms over the entire study
  - Conclusion: HPV screening leads to earlier detection of lesions

Rijkaart D.C. et al (2012) Lancet oncology, 13(1), pp. 78-88

POBASCAM trial

- Baseline screen
  - 14/96 CIN2 are HPV –
  - 9/159 CIN3 are HPV –
  - 2/12 cervical cancer are HPV –
- Therefore 9.4% of all relevant lesions (CIN2+) are HPV-!

Rijkaart D.C. et al (2012) Lancet oncology, 13(1), pp. 78-88

Primary HPV screening- triage

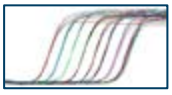
- Triaging HPV DNA positive samples
  - Good Cytology
    - Quality control!
  - HPV DNA typing
    - HPV16 and HPV18 have a higher risk
  - Protein markers
    - $p16^{INK4a}$
    - CINTec dual stain
      - $p16^{INK4a}$ together with Ki67

"Good" cytology

- Quality assessment
  - Internal and external quality assessment schemes
  - Guidelines on clinical behaviour
  - Can increase sensitivity and PPV
    - Diminish overtreatment
  - Need for trained cytotechnologists
    - Extremely difficult slides
    - Increase in screening time/slide

AML Experience

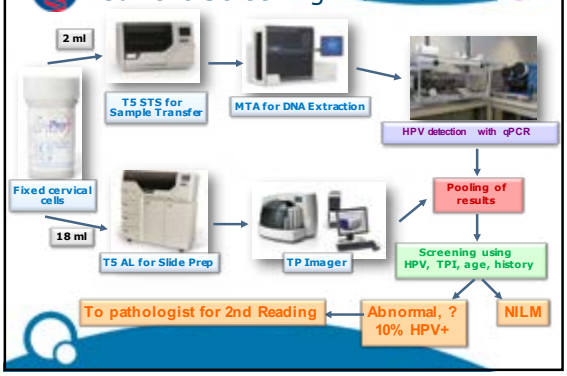
- Since 2006 HPV/cytology co-testing
  - HPV qPCR
    - Viral type and load
    - More than 900k cases



  - LBC cytology
    - BD SurePath 2006-13
    - Since June 2013 switched to Hologic Thinprep + Imaging
    - Automated cytology pre-screening using Imager

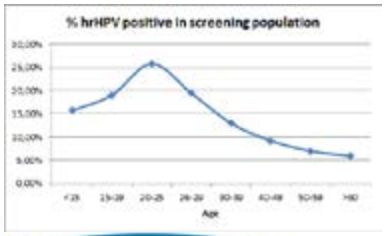
Micalessi I.M. (2012) Clin chem lab med, 50(4), pp. 655-61

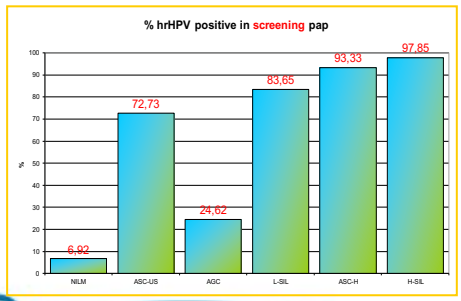
Current Screening Workflow



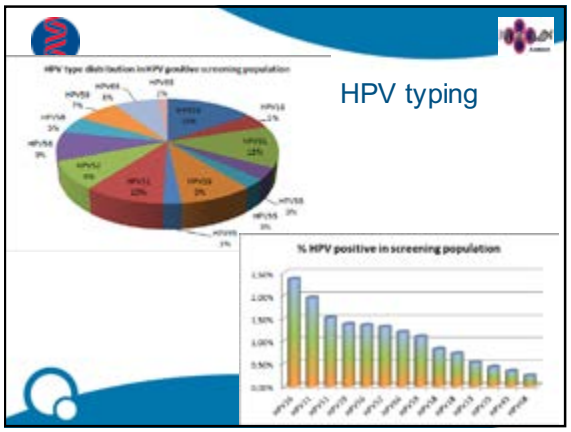
HPV positivity in screening pap

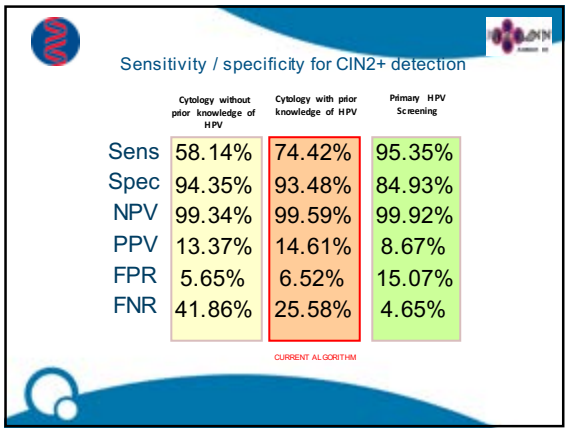
12,2% hrHPV positivity in screening population

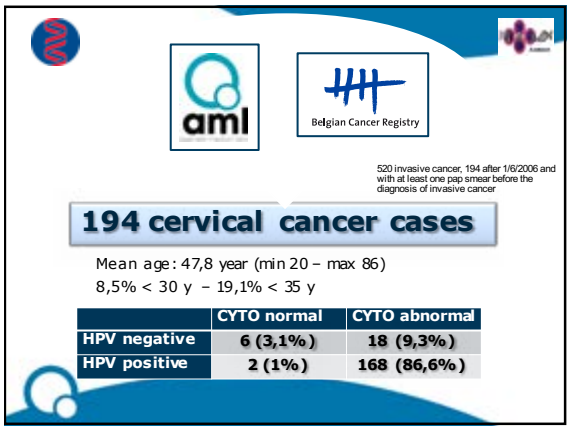


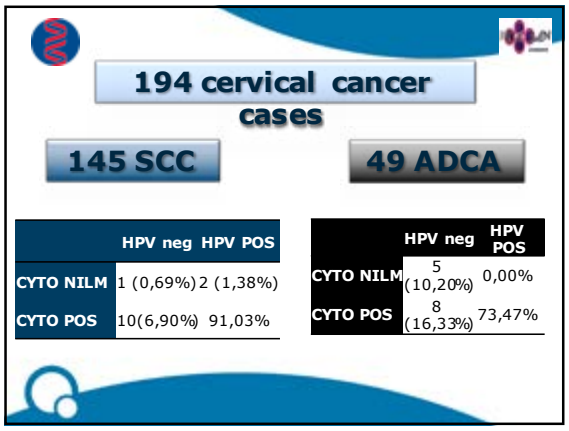


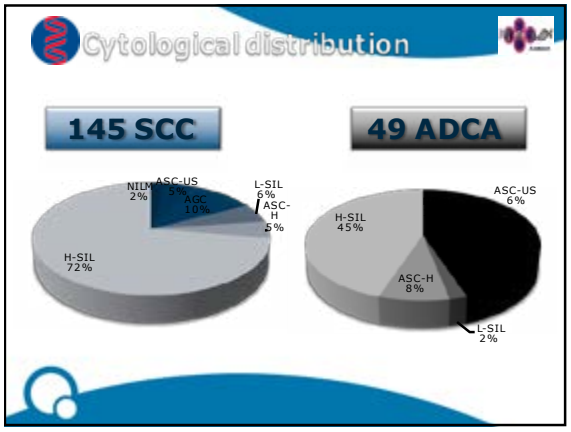
INVITED LECTURES

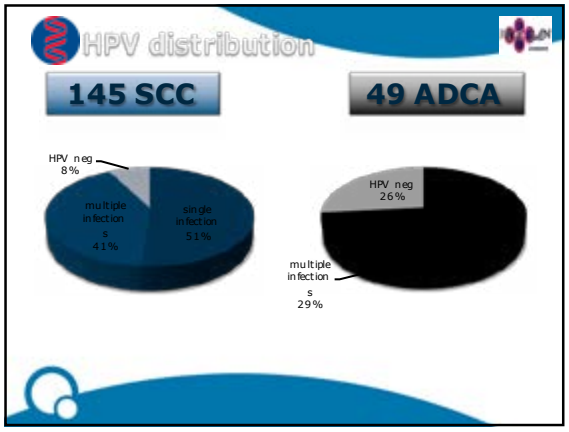


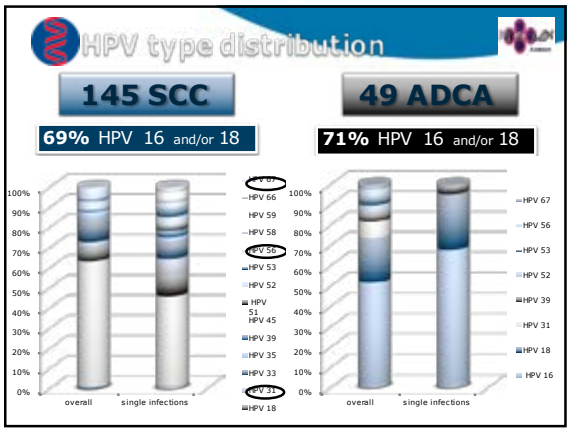












Conclusion combined HPV-cytology testing

- Inclusion of HPV testing in the primary round allows for extension of screening interval
  - 5 years? (POBASCAM)
  - 6 years? (extended ARTISTIC trial)
- Co-testing
  - Backup for problems with HPV testing
  - Increases sensitivity and detects additional lesions
  - Additional information
    - Infections
    - Adenocarcinoma (some endocervix, all endometrial)
    - Increases sensitivity and decreases FPR

Conclusion

- Increasing screening coverage will save lives!
- Cervical cancer is strongly associated with HPV infection
  - All cervical cancer?
- Most women get HPV infections, only a few get cervical cancer
  - "Cervical cancer is a very rare complication of a very common infection"
- The knowledge of the natural history of HPV infections can be used in primary and secondary prevention

Conclusion II

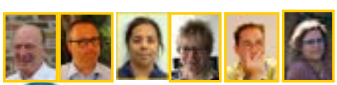
- In secondary prevention the inclusion of HPV detection can increase sensitivity
  - Be careful for false positivity and over-treatment (to comply with normative or expressed needs)
  - A Health Technology Assessment, including a local health needs assessment, is paramount before introduction
  - Awareness creation of the relative risk of HPV infections as well as adequate individual counseling by trained clinicians is important to reduce stress, and issues of 'victim blaming' that may impact the screening effectiveness

jpbogers@ambior.org

Acknowledgments

AML - Sonic Healthcare
Histopathology –Cytopathology-Molecular Diagnostics

apr G. Salembier
prof dr J. Bogers
dr S. Sahebali
dr I. Neetens
prof dr D. Vanden Broeck
dr apr I. Benoy



Emiel Vloorsstraat 9
2020 Antwerpen
Tel: 03 303 08 00
info@aml-lab.be

INVITED LECTURES

L 37

HPV DETECTION METHODS FOR CYTOTECHNOLOGISTS

Ina H BENOY
AML Department of Molecular Diagnostics, Sonic Healthcare, Antwerp, Belgium
AMBIOR Laboratory for Cell Biology & Histology, University of Antwerp, Antwerp, Belgium

Human Papillomavirus (HPV) is well known to be the principal cause of cervical cancer. It is the most common sexually transmitted virus. It has been estimated that around 70% of women get infected by the virus at some time point in their lives.

The development of cervical cancer can take place over a time period of about 10 to 15 years after HPV infection. It is therefore possible to diagnose premalignant lesions and treat patients successfully. The first step of prevention of this cancer is an appropriate screening program. With growing evidence, current screening recommendations to prevent cervical cancer are being revised towards primary HPV testing or co-testing. Besides identifying (wo)men at (cervical) cancer risk, HPV tests can also be used for several other distinct purposes: vaccine monitoring and epidemiology (assessing prevalence of type-specific HPV infections)

HPV's can not be cultured in vitro and no serological test is available which is sufficiently sensitive for reliable detection of HPV presence. Currently used HPV detection assays rely on the detection of viral nucleic acids (DNA or RNA) or viral proteins. The most widely used HPV DNA tests target a consecutive region of the viral genome. Using these consensus primers, the tests are potentially capable of detecting all mucosal HPV types. Most of these assays distinguish between high-risk and low-risk HPV types but are not designed for genotyping. HPV genotyping can be important for risk assessment, vaccination and follow-up.

RNA based HPV tests detect the viral mRNA. Their targets are usually the mRNA coding for the viral oncoproteins E6 and E7. These are over-expressed in severe lesions and cancer. Testing for E6/E7 mRNA aims to evaluate the presence of an active infection with cell-transforming potential, whereas HPV DNA assays detect only the presence of viral genomes.

Molecular-biology techniques used in HPV screening methods, can generally be classified into two categories: signal amplification assays and target amplification assays. Signal amplification methodologies directly increase the signal in proportion to the amount of target in the reaction. Target amplification multiplicates fragments of DNA/RNA from a targeted sequence. The most well-known example of this technique is the polymerase chain reaction (PCR).

Given the multitude of commercially available HPV tests, validated assays which assure high-quality screening need to be identified. VALGENT, a framework for HPV test comparison, allows verification of minimal criteria for use in cervical cancer screening. Continuous monitoring of test performance is necessarily to assure optimal safety of HPV based screening programs. Only clinically validated assays should be used.

An HPV assay for screening purposes should be reliable and reproducible, fast and robust, less hands-on time, highly automated, clinical validated and must be offered at an affordable cost. Unfortunately, the ideal HPV test does not exist (yet).

INVITED LECTURES

L 38

THE MOLECULAR CLASSIFICATION OF BREAST CANCER: IMPLICATIONS FOR THE SURGICAL PATHOLOGIST

Cecile Colpaert MD, PhD, GZA and UZA Antwerp

Introduction

Breast cancer is a heterogeneous disease at the morphologic, immunohistochemical and molecular level. The molecular studies have further confirmed this morphologic impression of heterogeneity and continue to improve our understanding. The molecular classification brought a paradigm shift in the treatment of breast cancer patients: breast cancer is no longer treated as one disease. Commercially offered multigene prognostic/predictive assays are increasingly used by oncologists in clinical decision making. As the molecular understanding of the disease increases, more targeted and hopefully less toxic therapies will become available.

Pathologists have started to pay more attention to biomarker evaluation, because almost all molecular studies showed distinct differences in hormone receptor (HR)-positive and HR-negative disease. Molecular classes do have morphoimmunohistologic correlates that can be applied in routine practice. The integration of novel biomarkers and routinely tested clinicopathological features, such as HR and human epidermal growth factor receptor 2 (HER2) status, may guide clinicians in systemic therapy decisions.

This presentation reviews the current state of molecular classification of breast cancer and the implications for the pathologists dealing with breast cancer in daily practice.

Molecular classification based on gene expression profiling: intrinsic molecular subtypes

A seminal discovery of breast cancer research over the last two decades was the description of the so-called intrinsic breast cancer subtypes (luminal, basal-like, HER2-enriched and normal-like) employing microarray-based gene expression profiling (Perou C et al. 2000), with the later subdivision of luminal tumours into A and B categories (Sorlie T et al. 2001). Extension of the molecular subtype studies by other groups resulted in the discovery of additional rare molecular subtypes, including claudin low, molecular apocrine and interferon rich (Prat A et al. 2010, Hu Z et al. 2006, Foulkes W et al. 2010, Farmer P et al. 2005).

Luminal Subtypes (60-70%)

Luminal A is the most common subtype of breast cancer. These tumours highly express ER-related genes and typically demonstrate ER+, PR+/-, HER2- and low Ki-67 proliferation index. This subtype includes invasive carcinoma no special type (NST) histological grade 1-2 , classic lobular, tubular, cribriform, mucinous and neuroendocrine carcinoma.

Luminal B subtype is less common than Luminal A. These tumours express ER (often low levels) with low or negative PR levels. They tend to be of higher histological grade with high expression levels of proliferation-related genes, thus demonstrating a higher Ki-67 proliferation index than Luminal A subtype. Some Luminal B tumours show HER2 amplification/overexpression. There is no universally accepted definition to discriminate Luminal A from Luminal B tumours: there is no absolute cutoff for the Ki-67 index to separate the 2 subtypes (14%? 20%?) and some tumours may be misclassified based on ER/HER2 profiles. Histological subtypes belonging to this group are invasive carcinoma NST grade 2-3 and micropapillary carcinoma.

HER2 enriched (15-20%)

This subtype shows high expression of genes in the HER2 amplicon and low expression of ER and related genes. Thus, the ER-, PR-, HER2+ immunohistochemical profile is commonly used as a surrogate for the HER2-enriched subtype. However, ER and HER2 status alone are not accurate surrogates for true intrinsic subtype status as some ER-/HER2+ tumours fall into the basal-like cluster (Parker J et al. 2009).

Histological subtypes belonging to this group are invasive carcinoma NST grade 2-3, apocrine carcinoma and pleomorphic lobular carcinoma.

Basal-like (15-20%)

Basal-like carcinomas highly express genes of basal epithelium and demonstrate low expression of ER-related genes and HER2. Thus, they typically show "triple-negative (TNBC)" phenotype: ER-, PR-, HER2-. These tumours are prevalent in younger women and in those harboring BRCA1 germline mutations. This group shows the greatest diversity with regard to histopathological features, molecular profiling, response to chemotherapy and survival outcomes. Analysis of gene expression profiles of 587 TNBC cases identified 6 subtypes with different outcomes and differing therapy response: 2 basal-like, an immunomodulatory, a mesenchymal, a mesenchymal stem–like and a luminal androgen receptor subtype (Lehman B et al, 2011).

Basal-type breast cancers are a subset of TNBC in which the tumours also express high molecular weight cytokeratins found in normal basal/myoepithelial breast cells, such as CK5/6 and CK14; p63 and epidermal growth factor receptor (EGFR) are also frequently expressed. These tumours are almost always high grade with pushing margin, central necrosis/fibrosis and extensive lymphocytic infiltrate. However, there has been increasing evidence that the definition of basal-like carcinoma based on morphology and expression of HR, HER2 and basal markers on the one hand, and gene expression profiling on the other hand, varies significantly and does not reproducibly identify the same lesion (Gazinska P et al. 2013). Therefore, subclassification of TNBC into basal-like and non-basal-like based on immunoexpression of basal markers is of limited value.
Histological subtypes belonging to this group are invasive carcinoma NST grade 3, medullary, metaplastic, adenoid cystic and secretory carcinoma.

Normal breast like

This subtype was so named because of higher expression of many genes known to be expressed by adipose tissue and other non-epithelial cell types. Some authorities have questioned the existence of this subtype and argue that the observed gene expression profile is derived from normal breast stroma, thus most likely representing artifacts of tissue procurement.

Other rare subtypes include claudin low, molecular apocrine and interferon rich. The biological and clinical significance of these subtypes remains to be determined.

Breast cancer intrinsic subtypes with prevalent immunohistochemical profiles and treatment options

Intrinsic subtype	Prevalent IHC	Treatment
Luminal A	ER+, PR+/-, HER2-, Ki-67 low	Endocrine therapy Chemotherapy for selected patients
Luminal B	ER+, PR+/-, HER2-, Ki-67 high	Endocrine therapy +/- chemotherapy
	ER+, PR+/-, HER2+, any Ki-67	Chemotherapy + anti-HER2 therapy + endocrine therapy
HER2-enriched	ER-, PR-, HER2+	Chemotherapy + anti-HER2 therapy
Basal-like	ER-, PR-, HER2-	Chemotherapy

Adapted from: Toss and Cristofanilli Breast Cancer Research (2015) 17:60

Gene expression based prognostic signatures

The rationale for developing multi-gene based prognostic tests is to add prognostic and predictive information to conventional biomarkers. MammaPrint, Oncotype DX and PAM50 are among the best validated and commercially available assays at this point of time.

MammaPrint (Amsterdam 70-Gene Profile)

One of the first commercially available and US Food and Drug Administration (FDA)-approved signatures was the 70-gene MammaPrint assay, which stratifies T1-T2N0 patients into low- or high-risk for distant metastases at 5 years. It is a supervised DNA microarray analysis initially developed for fresh frozen tissue, but recently also validated for formalin fixed paraffin embedded (FFPE) tissue. Moreover, on the same tissue used for MammaPrint, the BluePrint test can be performed. The latter is a molecular subtyping assay that analyzes

the mRNA levels of 80 additional genes to better discriminate among the molecular subtypes. Combining MammaPrint and BluePrint allows patients to be stratified into luminal-type/MammaPrint low-risk (similar to luminal A), luminal-type/MammaPrint high-risk (similar to luminal B), HER2-type and basal-type. This stratification has demonstrated several implications in neoadjuvant trials.

Oncotype DX

The 21-gene Oncotype DX assay was developed to estimate the risk of relapse in ER+, lymph node-negative breast cancer. Oncotype DX uses FFPE tumour tissue to divide patients into 3 groups based on their recurrence score (RS): low risk (RS of less than 18), intermediate risk (RS of 18 to 30), or high risk (RS of more than 31). Oncotype Dx is the only assay that achieved level IB evidence and has been incorporated into current National Comprehensive Cancer Network and American Society of Clinical Oncology guidelines, since it demonstrated a role as a predictive test in two prospectively designed retrospective studies with tumour specimens obtained from randomized clinical trials comparing tamoxifen with or without chemotherapy.

PAM50 (Prediction Analysis of Microarray (PAM50))

The FDA cleared PAM50 test defines the four major intrinsic subtypes of breast cancer through the analysis of 50 classifier genes and five control genes in fresh frozen or FFPE tumour tissue. Along with the identification of subtypes, PAM50 has been shown to be an independent predictor of survival in breast cancer (Chia S et al. 2012). PAM50 generates a numerical score (risk of recurrence, or ROR) that along with clinical features estimates the risk of relapse at 10 years in postmenopausal women with stage I/II node-negative or stage II node-positive (one to three positive lymph nodes) and HR-positive breast cancer (Filipits M et al. 2014). In patients with ER-positive and node-negative early breast cancer, the PAM50 platform has been demonstrated to provide more prognostic information than the Oncotype DX test, since PAM50 was better able to distinguish between intermediate- and high-risk patients (Dowsett M et al. 2013).

In the same study, PAM50 and Oncotype DX assays were compared with the IHC4 score, a prognostic model that combines quantitative IHC measures of ER, PR, HER2, and Ki-67. Relatively similar information was provided by ROR and IHC4 in all patients, but in the HER2-negative/node-negative subgroup, ROR was more informative than IHC4 .

The evaluation and the comparison between the predictive value of PAM50 and Oncotype DX are ongoing in the prospective RxPONDER trial (NCT01272037), and results from the prospective MINDACT (NCT00433589) and TAILORx (NCT00310180) trials are awaited to have direct evidence of the predictive value in the adjuvant setting of, respectively, MammaPrint and Oncotype DX assays. Results are not to be expected before the end of 2015/2016.

The Belgian KCE recently recommended that the reproducibility and reliability of the IHC4 assay should be further explored in order to facilitate wider acceptance and use of this more economical testing option.

Multi gene signature	Company	Tissue requirements	Technique	Number of Genes	Output/score
Mammaprint	Agendia	Fresh frozen or FFPE	Microarray	70	Low versus High risk
Oncotype DX	Genomic Health	FFPE	qRT-PCR	21	RS score: Low versus Intermediate versus High
Prosigna (PAM 50)	Nanostring	FFPE	Multiplexed measurement of mRNA via hybridization	50	ROR score: Low versus Intermediate versus High

Next generation sequencing (NGS)

The revolution in next-generation sequencing has enabled genome sequencing studies on hundreds of individual human malignancies, including breast cancer. These techniques identify somatic DNA changes in the cancer through comparing DNA sequences of the primary tumour with the patient's normal tissue. Stratifying patients according to some of the increasing number of genetic aberrations identified in the hormone sensitive, HER2+ and triple negative tumour groups is transforming the design of a new generation of studies. Key genetic mutations are found across all sub-groups of the disease, but at widely differing frequencies. Mutations in PIK3CA (phosphoinositide 3-kinase catalytic subunit) are most frequent in ER+ disease; they are found in around 45% of Luminal A tumours. PIK3CA inhibitors are already being tested in early phase clinical trials. The PI3K pathway is also thought to play a role in HER2+ disease with the potential to mediate resistance to anti-HER2 therapy. About 75% of HER2+ tumours and 80% of basal-like tumours carry mutations in TP53.

The application of NGS in the metastatic setting holds promise in further advancing precision medicine. Patients with a breast cancer diagnosis can now benefit from a number of novel targeted therapies that are either FDA-approved or under development and that have already translated to significant improvement in survival for this disease.

FURTHER READING:

Toss A and Cristofanilli M, Breast Cancer Research (2015) 17:60-71

Vuong D et al, Virchows Arch (2014) 465:1–14

Ellis M, Perou C. The genomic landscape of breast cancer as a therapeutic roadmap. Cancer Discov. (2013) 3:27-34.

L 39

NEW BIOPSY TECHNIQUES IN LUNG PATHOLOGY

Weynand Birgit, Ocak Sebahatµ, Delos Monique.*
Pathology department, UZ Leuven, Leuven*
Pneumology departmentµ and Pathology department, CHU Dinant Godinne, Yvoir
Birgit.Weynand@uzleuven.be

I-Introduction

Lung biopsies whether taken for the subtyping of an interstitial lung disease or for the diagnosis of a lung cancer need to be as adequate as possible.

In the first case, a surgical biopsy is the gold-standard to obtain a definite diagnosis, with as consequence a possible treatment, but most of all to determine the prognosis of the patient. In many cases, however, these patients have a very bad lung function precluding such an intervention.

In the second case, molecular analysis of tumor tissue is necessary to determine if a patient with a non-small cell carcinoma is eligible for a targeted therapy. Therefore a biopsy with a sufficient amount of tumor is mandatory not only or the diagnosis, but also to allow for ancillary techniques.

In the last years, new biopsy techniques have emerged. We will discuss cryobiopsies (ERBE, Zaventem, Belgique) taken during endobronchial endoscopy and trans-thoracic biopsies taken with large needles such as Spirotome (Medinvents, Wellen, Belgique).

II-Cryobiopsy

This endoscopic technique is used for the diagnosis of a tumor or for the subtyping of an interstitial lung disease. The cryoprobe (2.4 mm-10,5G or 1.9mm-12,5G) is introduced via an endoscope and placed in close contact with the area to be biopsied. After a few seconds of freezing, the hardened tissue can easily be removed. Cryobiopsies are well preserved tissue fragments, with less crush artifacts in comparison with the classical forceps biopsies. The accuracy of the diagnosis is better and the technique is less invasive with less complications in comparison with a surgical biopsy. The main disadvantage is the fact that a sedation or a general anesthesia is necessary during the examination. The patient needs to be intubated because the biopsy fragment obtained is too large to be removed within the endoscopy channel. The endoscope needs therefore to be taken out in whole after each biopsy. Contra indications include severe hypoxemia, severe asthma, not cooperating patients, a massive hemoptysis and a not corrected coagulation disorder.

Several recent publications have demonstrated that this technique is safe and assures a better diagnosis in lung cancer pathology.

In interstitial lung diseases, the technique is safe and reliable, as well as less expensive than a surgical biopsy. In most cases, where the surgical approach is impossible, this technique allows to diagnose accurately the interstitial lung disease and to prevent the acute exacerbation often observed in the early postoperative period. Nevertheless, the small size of the cryobiopsy in comparison with a surgical biopsy needs a particularly experienced pathologist.

III- Spirotome

The Spirotome is a needle provided with a trocar which is placed trans-thoracally under CT-guidance. The stylet is removed and replaced by the needle. The needle has a twisted loop, like a cork-screw which enables the penetration into the tissue and gives a particular aspect to the biopsy. The size of the needle is 14G, with a diameter of 2 mm, in comparison with the 20 to 21G (1mm) of the Nordenström needles (Rotex), classically used for a trans-thoracic approach. The main interest of this needle is the study of peripherally located lung nodules which cannot be diagnosed with a conventional endoscopic approach. The morbidity, mortality and cost of this technique in comparison with a surgical biopsy are much lesser. Contra indications include severe emphysema, a bad lung function, a tumor measuring less than 1 cm (relative contra indication) and a hyper-vascularized tumor. In a retrospective study done by our group comparing the Spirotome with the Rotex needle in a group of 100 patients, we observed a mean biopsy size of 15 mm (2-50 mm). Only cytological material could be obtained with the Rotex needle insufficient for the preparation of paraffin-embedded cell blocks.

The main advantages of the Spirotome are a better characterization of benign and malignant lesions, sufficient material to allow molecular analyses in the context of a malignant tumour without being responsible for a higher rate of complications. The classical complications observed by a trans-thoracic approach being hemoptysis and pneumothorax, whatever technique applied.

IV-Conclusion

New biopsy techniques have been developed in recent years either for an endoscopic or a trans-thoracic approach. They are safe and reliable with limited complications.

In tumor diagnosis, they allow to obtain sufficient material for a definite diagnosis and for ancillary techniques.

In interstitial lung diseases, they reduce the need for surgical biopsies, but are a major challenge for the experienced pathologist.

References

1. Hagmeyer L, Theegarten D, Wohlschläger J, Treml M, Matthes S, Priegnitz C, Randerath WJ. The role of transbronchial cryobiopsy and surgical lung biopsy in the diagnostic algorithm of interstitial lung disease. Clin Respir J 2015; xxx:xxx-xxx. doi:10.1111/crj.12261.
2. Hernandez-Gonzalez F, Lucena CM, Ramirez J, Sanchez M, Jimenez MJ, Xaubet A, Sellares J, Aguti C. Cryobiopsy in the diagnosis of diffuse interstitial lung disease: diagnostic yield and cost-effectiveness analysis. Arch bronconeumol 2015; 51:261-7.
3. Hetzel J, Eberhardt R, Herth FJF, Petermann C, Reichle G, Freitag L, Dobbertin I, Franke KJ, Stanzel F, Beyer T, Möller P, Fritz P, Ott G, Schnabel PA, Kastendieck H, Lang W, Morresi-Hauf AT, Szyrach MN, Muche R, Shah PL, Babiak A, Hetzel M. Cryobiospy increases the diagnostic yield of endobronchial biopsy: a multicentre trial. Eur Respir J 2012; 39:685-690.
4. Ocak S, Duplaquet F, Jamart J, Weynand B, Delos M, Eucher P, Dupont M, Delaunois L, Sibille Y, Dahlqvist C. Diagnostic accuracy and safety of a novel frontal core biopsy device in CT-guided percutaneous transthoracic biopsies of pulmonary and pleural lesions. Proceedings of the 3rd European Congress for Bronchology and Interventional Pulmonology (ECBIP) 2015; 23: O-10. Barcelona, Spain.
5. Ocak S, Duplaquet F, Weynand B, Delos M, Jamart J, Eucher P, Delaunois L, Sibille Y, Dahlqvist C. CT-guided transthoracic biopsies of lung lesions: diagnostic accuracy and safety of a novel frontal core biopsy device. Eur Respir J 2013; 42: Suppl. 57, 472s (P2317).

INVITED LECTURES

L 40

The pathology of bowel cancer screening

Maurice B Loughrey[1] & Neil A Shepherd[2]
[1]*Department of Histopathology, Royal Victoria Hospital, Belfast, UK, and* [2]*Gloucestershire Cellular Pathology Laboratory, Cheltenham General Hospital, Cheltenham, Gloucestershire, UK*

Loughrey M B, Shepherd N A
(2014) *Histopathology*

The pathology of bowel cancer screening

Colorectal cancer screening is widely promulgated in many parts of the world and population screening is occurring in many countries, especially in western Europe. Although, intuitively, it might be thought that the pathology resulting from screening should be straightforward, being mainly that of polyp diagnosis and the biopsy diagnosis and staging of established adenocarcinoma, in fact experience has shown that there are several areas of considerable difficulty and controversy. In the UK somewhat different programmes, all based on faecal occult blood (FOB) screening, have been developed and each has generated similar pathological conundrums. These include the biopsy diagnosis of adenocarcinoma, colorectal serrated pathology, the diagnosis and management of polyp cancers and last, but certainly not least, the phenomenon of the large sigmoid colonic adenomatous polyp with epithelial misplacement/pseudo-invasion. Polyp cancers provide especially difficult management conundrums and discussion of that management within a multidisciplinary team-based management meeting is regarded as essential in the UK. Large adenomatous polyps of the sigmoid colon with epithelial misplacement are selected into FOB-based screening programmes and have provided extraordinary diagnostic challenges. Finally, the quality assurance procedures introduced for screening can ensure a considerable overall improvement in the quality of lower gastrointestinal tract pathological reporting.

Keywords: adenoma, colorectal cancer, epithelial misplacement, polyp cancer, pseudo-invasion, screening, serrated pathology

Introduction

Colorectal cancer (CRC) provides an ideal disease for population screening, fulfilling all the requirements demanded before a screening programme is considered.[1] It is a common disease, representing the third most commonly diagnosed cancer worldwide and the second most common cause of cancer-related death, for men and women, in developed nations. It thus presents a significant healthcare burden globally, one which is likely to increase if developing nations adopt the 'western diet'. CRC has an identifiable precursor, the adenoma or adenomatous polyp, and the natural history of the disease is well understood. The concept that CRC evolves from a precursor lesion was based on studies from the Pathology Department of St Mark's Hospital in London in the 1920s, which laid the foundation for subsequent research ultimately describing the adenoma–carcinoma sequence published in 1975.[2,3] It is now accepted that the vast majority of CRCs arise from such adenomas. Moreover, the progression from adenoma to carcinoma, which is likely to take place in a very small number of polyps, probably occurs over approximately a 10-year period, the polyp 'dwell time', allowing a generous window of opportunity for effective screening.[4] A variety of potential screening tests have been developed which demonstrably can identify at least some CRCs at an early stage before symptoms have devel-

Address for correspondence: Professor N A Shepherd, Gastrointestinal Pathology, Gloucestershire Cellular Pathology Laboratory, Cheltenham General Hospital, Sandford Road, Cheltenham, GL53 7AN, UK. e-mail: neil.shepherd@glos.nhs.uk

oped and when local treatment alone can be considered. Furthermore, some of these tests can also detect and permit the removal of colorectal adenomas, thereby preventing their development to CRC.

This paper describes the development of bowel cancer screening (BCS) programmes worldwide, with a focus on pathology-related issues and the specimens and diagnoses encountered, emphasizing the major difficulties encountered in routine BCS pathology practise.

The history of bowel cancer screening

In 1967, Greegor reported that early-stage CRC in asymptomatic patients could be detected in his office-based practise with the use of faecal occult blood (FOB) guaiac test cards.[5] In 1968, the World Health Organization defined the first set of principles recommended to underpin population screening programmes.[1] Colonoscopy and flexible sigmoidoscopy were both introduced in the 1970s, with significant enhancements in instrumentation to follow over subsequent decades. Before the availability of colonoscopy, a positive FOB test had to be investigated by double-contrast barium enema which, even today, is of limited sensitivity, especially for adenomas <1 cm.[6] These developments were all pivotal in allowing consideration of population-based screening for CRC.

The evidence base for home FOB-based CRC screening stems from three high-quality randomized controlled trials (RCT) demonstrating significant reductions in cancer-related mortality over prolonged follow-up.[7–9] Immunochemical FOB testing, which tests for globin rather than the haem detected by the guaiac method, has been developed as an alternative to guaiac testing in more recent years and, although there is no RCT evidence demonstrating reduced CRC mortality using this screening test, numerous studies have consistently demonstrated enhanced sensitivity and specificity for both advanced adenomas and CRC, compared to guaiac testing, without loss of specificity.[10] Immunochemical FOB testing is now favoured by the American College of Gastroenterology.[11]

There is good evidence from three RCTs for reduced CRC mortality from screening with flexible sigmoidoscopy, namely the Telemark and NORCCAP studies in Norway, and a large UK study which examined one-off sigmoidoscopy alone as the screening intervention in 57 237 individuals.[12–14] This latter study demonstrated a 31% reduction in CRC mortality in the screened group compared to controls.[14] Less evidence is available to support a role for primary colonoscopy screening. Observational studies, for example the National Polyp Study, have demonstrated a significant reduction in observed versus expected CRCs in the study cohort compared to the control group after baseline clearing colonoscopy.[15] Case–control studies have also reported a significant reduction in CRC mortality and advanced neoplasia detection rates.[16,17] Some of these studies have suggested that colonoscopy might be less effective in detecting right-sided colonic cancers, although this may be related to the quality of colonoscopy. Although more evidence is awaited to support colonoscopy as a primary screening tool, it has an established role as the follow-up investigation in strategies employing other primary screening modalities.

This menu of options for CRC screening makes it much more complex than screening for other cancers, such as breast cancer. Which screening test or tests to adopt for CRC depends on a variety of factors, principally around economics, the nature of the healthcare system available to the population in question, and the available evidence at the time of programme initiation. The resultant pathology specimens generated will reflect, both in volume and type, the screening test or strategy chosen. In general, FOB-based programmes yield more advanced stage cancers and larger polyps than primary endoscopy screening. Endoscopy screening generates many more small polyps, probably of little or no relevance to an individual's subsequent CRC risk.

The most common population screening strategy uses a home guaiac FOB detection kit to select individuals for follow-up colonoscopy to detect and remove any polyps found or to diagnose any underlying malignancy. This is the basis of national programmes in the UK, in many other European countries and in Australia.[18] CRC screening programmes in the United States vary widely state by state, with a wide range of strategies employed, and primary colonoscopy screening particularly popular, usually offered to individuals aged 50 years or older.[19]

Pathological diagnoses in UK population-based bowel cancer screening

Endoscopic specimens generated through BCS colonoscopy must be clearly marked as such to the reporting pathologist, and this information will enable capture of resultant pathology diagnoses for analysis. By way of example, Table 1 summarizes all histological diagnoses relating to 5857 endoscopy specimens

(from 2452 colonoscopies), obtained from the inception of the Northern Ireland BCS Programme in 2010 through to 2013.

Four percent of specimens in this series yielded a diagnosis of adenocarcinoma, 26% of which were within polyps at endoscopy. Together, tubular and tubulovillous adenomas accounted for more than 60% of all diagnoses, by far the most common polyp diagnosed. Many of these adenomas were large and sigmoid colonic in location (data not shown). Hyperplastic polyps were the next most common polyp, representing 16% of all diagnoses. Other serrated family polyps accounted for approximately 2% of diagnoses in this series, possibly under-represented as a result of evolving diagnostic thresholds for this entity since the inception of the programme. All other benign polyp diagnoses were relatively rare, the next most common being the 'inflammatory polyp'. These are a heterogeneous group, and may be encountered in the setting of chronic inflammatory bowel disease, diverticulosis or mucosal prolapse. In the setting of prominent mesenchymal change with minimal associated inflammation and no glandular serrations or neoplasia, the term 'post-inflammatory polyp' is preferable. Other polyps, such as those demonstrating hamartomatous or neuro-endocrine (carcinoid) features, are extremely infrequent in BCS pathology. Stromal polypoid lesions, also rare in the setting of BCS, are discussed by Voltaggio and Montgomery in the accompanying paper within this annual Review issue. The most common non-polyp diagnosis within BCS endoscopic specimens is 'inflammation', usually related to inflammatory mucosal diseases, including chronic idiopathic inflammatory bowel disease, ischaemic colitis or so-called diverticular colitis.

Table 1. Histological diagnoses for 5857 endoscopy specimens from the Northern Ireland bowel cancer screening programme

Diagnosis	Numbers	% of Total
Adenocarcinoma	175	3.0
Adenocarcinoma (in polyp)	61	1.0
Tubular adenoma	2765	47.2
Tubulovillous adenoma	1032	17.6
Villous adenoma	19	0.3
Hyperplastic polyp	940	16.0
Sessile serrated lesion	113	1.9
Traditional serrated adenoma	23	0.4
Inflammatory polyp	35	0.6
Inflammation	253	4.3
Normal	326	5.6
Other	115	2.0
Total	5857	100

Extrapolating these data to the English BCS programme (and the proportions of polyp diagnoses and cancer prevalence are very similar to the Northern Irish data), in which approximately 70 000 cancers have been detected to date, gives magnitude to the problems relating to adenomas with epithelial misplacement and to polyp cancers (diagnosis and management). In our experience of reporting BCS pathology cases, both primarily and as referral cases for second opinion, these are by far the most common problem areas. Other recurring issues causing difficulty are less frequent but still significant. In this study, we discuss four of these areas in varying detail:

- The large sigmoid colonic adenomatous polyp and epithelial misplacement
- Reporting stage pT1 adenocarcinomas ('polyp cancers')
- Minimal criteria for the biopsy diagnosis of adenocarcinoma
- Serrated pathology.

The large sigmoid colonic adenomatous polyp and epithelial misplacement

The data presented above demonstrate that the adenoma is overwhelmingly the most common type of polyp identified by BCS programmes, constituting more than 60% of all polyps detected in UK population-based BCS programmes. The sigmoid colon is the most common site for BCS-detected adenomas and, furthermore, sigmoid colonic adenomas tend to be significantly larger than those encountered elsewhere in the colon and rectum. Many of these sigmoid colonic adenomas are pedunculated and tend to tort, ulcerate and bleed, causing a positive FOB test, and thereby such larger sigmoid colonic adenomas are preferentially selected into FOB-based BCS programmes.

A common and important pathological feature, seen most commonly in large sigmoid colonic adenoma polypectomy specimens, is that of epithelial misplacement. This is a phenomenon whereby epithe-

lium, usually accompanied by lamina propria, is relocated into the submucosa of the gastrointestinal tract. It is encountered most often in the sigmoid colon as a sequela of repeated mechanical trauma to large polyps, resulting from a combination of factors: the narrow, highly motile sigmoid colon, solid faecal material at this distal location, and the frequent association with diverticulosis, particularly in the age range of BCS (60–75 years), leading to a reduced lumen as a result of muscular hypertrophy and enhanced trauma to sigmoid colonic polyps (Figure 1). It is likely that mucosal prolapse also plays a key role in its pathogenesis, as a characteristic accompaniment is fibromuscular proliferation in the lamina propria, between adenomatous crypts.[20] In UK BCS, at least 85% of polyps demonstrating epithelial misplacement are sigmoid colonic in location.

Probably the most challenging diagnostic difficulty facing BCS pathologists lies in distinguishing epithelial misplacement from invasive adenocarcinoma (Figure 2; Table 2). Despite this being a common and well-recognized phenomenon, this issue has caused considerable diagnostic problems for decades and continues to do so.[20,21] In UK BCS programmes, it has triggered mandatory double reporting of all stage pT1 colorectal cancers. A BCS 'Expert Board' has been convened in the UK centred on, and financed by, the English programme, to deal with such cases. Since its establishment in 2009, more than 200 cases have been assessed by this Board. Indeed, the constitution insists on three 'expert' gastrointestinal pathologists to ensure a majority diagnosis, as agreement is by no means universal between the three, emphasizing the diagnostic difficulties associated with epithelial misplacement. Further, the consensus diagnosis of the Board has been diametrically opposite to that of the referring pathologist(s) in about 30% of cases.

Although a confident diagnosis should be possible in most cases, in some the overlapping features make confident classification as benign or malignant simply impossible. Cases demonstrating the classical epithelial misplacement features of lobulated glands, lamina propria accompaniment, haemosiderin deposition and muscular proliferation of prolapse typically provide no diagnostic difficulties but many cases do not show all these classical features, or not in isolation. Mimicry is particularly a problem when glands

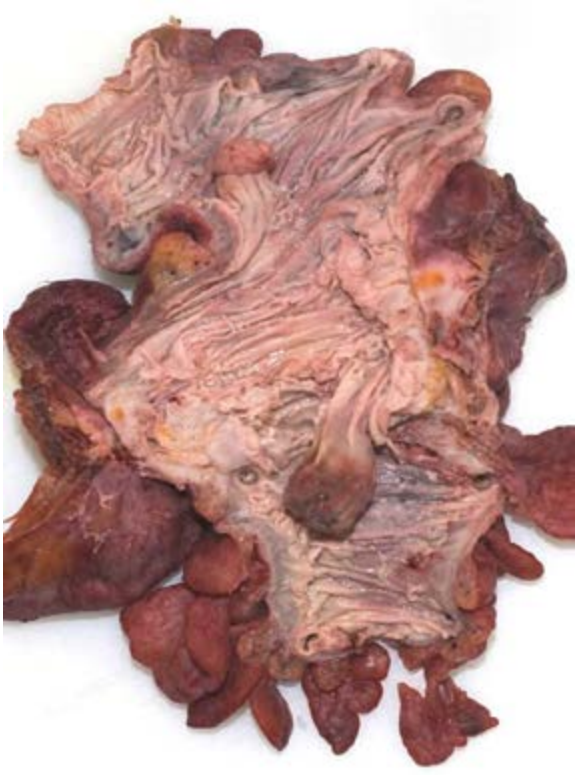


Figure 1. A sigmoid colonic resection undertaken for complicated diverticular disease. There are two lobulated adenomatous polyps. The upper polyp is relatively unaffected but the polyp present centrally shows deep congestion and is notably irregular and nodular. These are the characteristic macroscopic pathological features of large sigmoid colonic adenomatous polyps with epithelial misplacement.

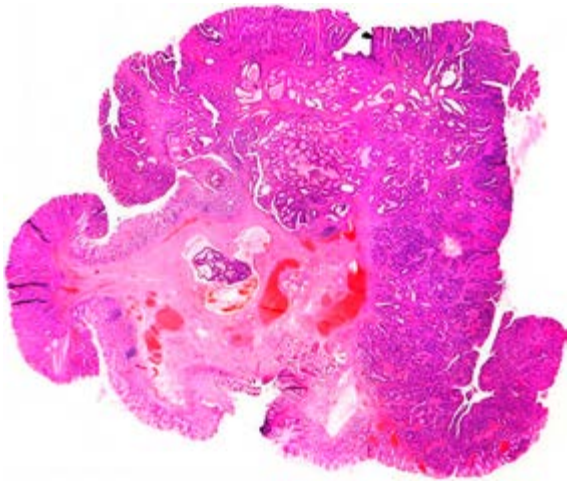


Figure 2. A large sigmoid colonic adenomatous polyp showing both epithelial misplacement and adenocarcinoma. The classical epithelial misplacement is best appreciated on the left with a mucinous pool in the submucosa and the adenomatous component appearing similar, with accompanying lamina propria, to that of the surface. To the right is a well differentiated adenocarcinoma. The histological similarity, even at this low power, is apparent.

Table 2. A comparison of the pathological features that may be valuable in differentiating epithelial misplacement from invasive adenocarcinoma

	Epithelial misplacement (EM)	Adenocarcinoma
Epithelial 'differentiation'	Usually similar to that of the surface adenomatous component	Variable and usually different to the surface adenomatous component
Lamina propria accompaniment	Characteristic but may be lacking when there is secondary inflammation and epithelial destruction	Usually absent. Can be present in rare, very well-differentiated carcinoma
Accompaniment by non-adenomatous epithelium	Characteristically seen when EM is due to previous intervention	Absent
Haemosiderin deposition	Characteristic and indicative of previous necrosis and/or haemorrhage	Usually absent
Mucosal prolapse changes	Often present	Usually absent
Mucus cysts	Characteristic. They probably represent epithelial misplacement that has become 'detached' from the more superficial components	Only present, usually, in mucinous tumours
Continuity with surface adenomatous component	Characteristic but often only appreciated in multiple levels and/or 3D reconstruction studies	Usually absent but some cases do show continuity, even in 3D reconstruction studies.
Involvement of muscularis propria (MP)	Usually absent. Can be seen very rarely, especially after previous intervention	Present if at least pT2
Budding	Usually absent but a similar phenomenon can be seen as a result of epithelial destruction and/or inflammation	Often present
Desmoplastic reaction to glands	Usually absent but fibromuscular stromal proliferation can accompany EM	Usually present
Lymphatic and/or vascular invasion	Absent	Diagnostic of cancer

showing high-grade dysplasia are misplaced and when epithelial misplacement is associated with rupture, mucin extravasation, secondary inflammatory changes, epithelial disruption and stromal reaction.[20] Distinguishing these potentially benign features from true stromal invasion can be a significant challenge.

We find a low-power view of most benefit in this distinction, to evaluate if the architecture of the misplaced glands is organoid or haphazard. Continuity between surface and deeper glands (which may only be apparent on examining multiple levels or using 3D reconstruction), misplaced adenomatous epithelium accompanied by lamina propria and non-adenomatous epithelium (Figure 3) and accompanying muscular proliferation of the type associated with mucosal prolapse (Figure 4), are features which favour benignity. On higher power, glandular angulation, single cell infiltration ('tumour budding') and convincing desmoplastic stromal reaction are all features which support a diagnosis of malignancy. One should look carefully for subtle lymphatic or venous invasion at and beyond the edge of the lesion, as identification of either would clearly confirm malignancy.

It is important to be aware of the potential pitfalls associated with previous biopsy or partial polypectomy. These can cause ulceration with acute mucosal necrosis, marked regenerative atypia and a desmoplastic stromal reaction, which may be difficult to distinguish from adenocarcinoma. One should therefore exercise caution before diagnosing adenocarcinoma in this setting. A feature which appears to be characteristic of the epithelial misplacement that accompanies previous endoscopic intervention is that non-neoplastic epithelium is also demonstrated in the submucosa, along with adenomatous epithelium.[20] Finally, in a small minority of cases, the Expert Board

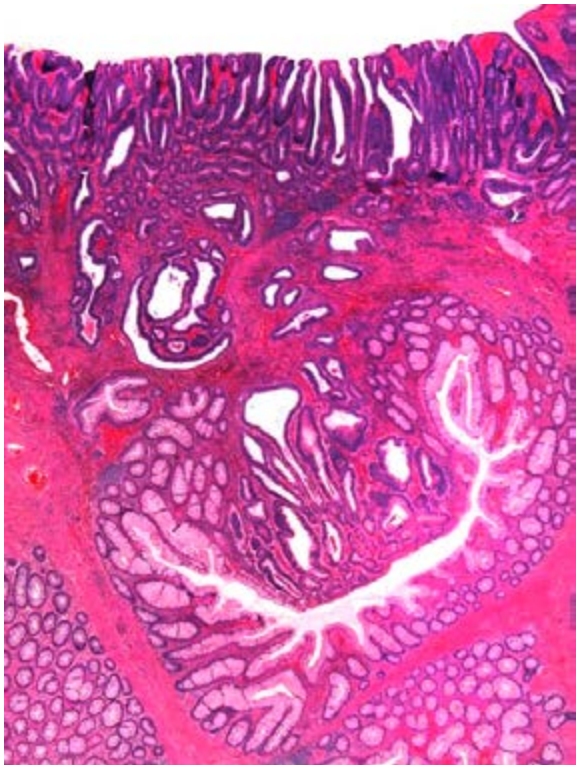


Figure 3. This sigmoid colonic polyp shows epithelial misplacement of both adenomatous and non-adenomatous epithelium. Although this can be seen as a result of the mechanical effects of torsion, it is an especial feature of epithelial misplacement induced by previous instrumentation, both endoscopic and surgical.

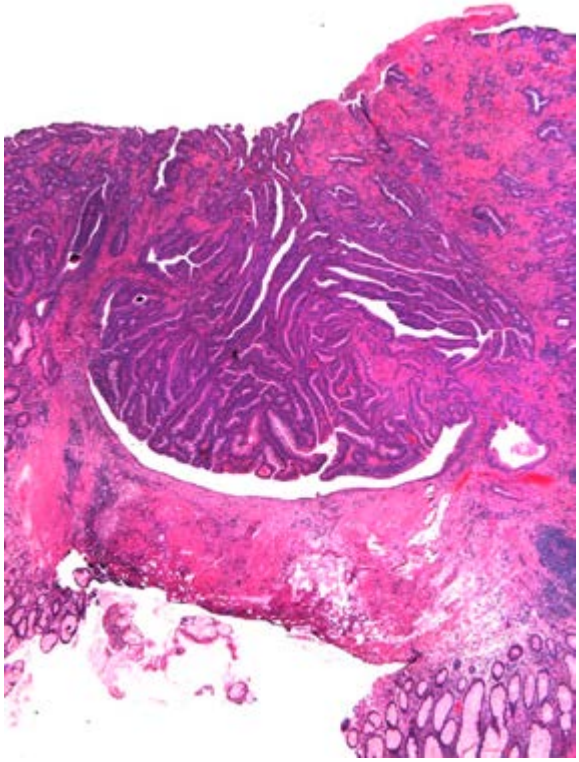


Figure 4. A large sigmoid colonic adenomatous polyp with two features characteristic of the mechanical changes to which these are subject. First, there is inversion of the surface adenomatous component such that its 'surface' faces the deep diathermy mark at the lower margin. Secondly, there is dramatic muscular proliferation (appearing pink here) between the adenomatous crypts in the superficial and deep components indicating the effect of mucosal prolapse.

and others have seen the coincidence of both epithelial misplacement and invasive adenocarcinoma (Figure 2), but it is emphasized that this is rare.

The difficulty in making a morphological distinction between epithelial misplacement and adenocarcinoma on haematoxylin and eosin-stained sections, alone, has encouraged attempts at developing adjuvant diagnostic tools. Although immunohistochemical analysis, including the use of antibodies to collagen IV, E-cadherin, MMP-1, p53 and stromelysin-3, has been reported to be of potential value, it is likely that the benefit of these adjuncts is least when the morphological features are particularly challenging.[20,22–25] Although in early development, we have found some utility in methods such as infra-red spectroscopy and 3D reconstruction. If continuity of epithelium of the superficial and deeper components is a particular feature of epithelial misplacement, then 3D reconstruction should be able to demonstrate this feature convincingly. Our initial studies have been interesting in that regard although, tantalizingly, we have shown evidence that polyp cancers also appear to demonstrate good epithelial continuity between the superficial and deeper components.[20]

Reporting stage pT1 adenocarcinomas ('polyp cancers')

Once a diagnosis of adenocarcinoma is made within a polypectomy specimen, the next problem facing the reporting pathologist relates to evaluating the morphological features within the cancer to determine whether or not further treatment is required, usually in the form of surgical intervention. Patients with locally excised stage pT1 CRC are at low but definite risk of residual disease in regional nodes, which could be cured by surgical intervention. Balancing the risks of surgery versus those of lymph node metastatic disease is the challenge, the goal being to offer surgery

to those most at risk of regional disease and avoid surgery in those with minimal risk.[26] Location of the pT1 cancer is important, as morbidity risk from surgery varies considerably by anatomical site.

Many studies have examined the morphological tumour features predictive of an adverse outcome in the form of residual disease, regional lymph node metastasis or distant metastatic recurrence, but unfortunately the evidence base is limited, generally featuring studies with small case numbers and often conflicting conclusions with regard to the most important features. Traditional features considered most predictive of adverse outcome in stage pT1 cancers are depth of invasion, poor differentiation, lymphovascular invasion and incomplete excision.[27,28] A wide variety of methods has been described for the evaluation of depth of tumour invasion.[29–31] Recent studies have examined other potentially useful features, such as tumour budding (Figure 5).[31–34] A detailed discussion of these features is beyond the scope of this review but is available elsewhere, in the form of a meta-analysis.[35] The guidance for reporting CRC in local excision specimens contained within the recently revised Royal College of Pathologists (UK) Dataset for Colorectal Cancer is recommended.[36]

Given the much higher frequency of such early-stage CRCs within the setting of BCS, compared to the symptomatic population, this is a common and important scenario for BCS pathologists and it is of paramount importance that they are familiar with, and report accurately, all morphological features which may be used to inform management and prognosis. To this end, use of a reporting data set, such as those offered by the Royal College of Pathologists (UK) and the College of American Pathologists (CAP, USA), is strongly advised, to ensure inclusion of all relevant or potentially relevant parameters. It should be noted that some features, for which evidence is evolving but not currently specified as mandatory reporting items, may be worth reporting in addition, if of interest to the local CRC multidisciplinary team meeting discussing management. As noted above, UK BCS programmes now mandate double reporting by BCS pathologists of all screen-detected stage pT1 CRCs. This should apply not only to the primary diagnosis but to all the pathological features reported. BCS pathologists should have access to a referral network for specialist opinion on both primary diagnosis and ancillary features, given their potential importance with respect to patient management.

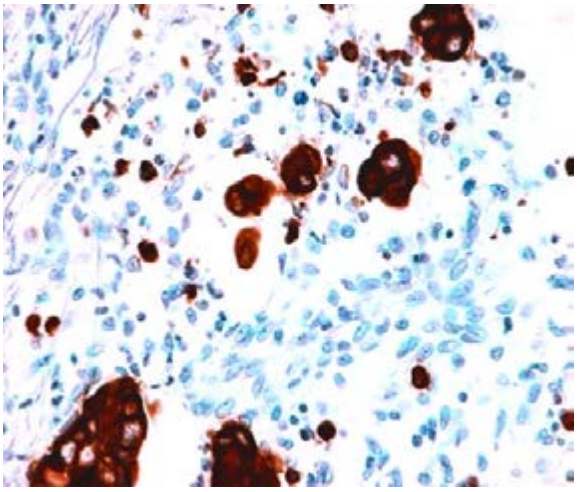


Figure 5. A polyp cancer with prominent epithelial budding, shown here by cytokeratin immunohistochemistry. This was the only adverse prognostic feature in the polyp cancer and yet, at the time of resection, there was lymph node involvement.

The importance of receiving polypectomy specimens intact, rather than piecemeal, needs to be emphasized to endoscopists, particularly if there are suspicious endoscopic features such as abnormal vasculature or 'non-lifting'. Piecemeal resection of large adenomas is less problematic, as completeness of excision is best assessed on endoscopic impression, but a piecemeal resection of an early-stage CRC makes accurate assessment of margin involvement and depth of invasion (by any method) impossible.[30,37] Pathologists should report what is assessable and indicate what is not. If an endoscopist considers they will be unable to remove such a lesion intact, especially in the allotted time within a busy BCS colonoscopy list, it may be preferable to defer removal to another dedicated time or to refer to an endoscopist with greater expertise in therapeutic polypectomy.[38,39] In this situation, biopsying the polyp at initial endoscopy is ill-advised, as resultant scarring may further tether the polyp to the submucosa and make local removal more difficult.[40,41]

Optimal specimen handling is essential for adequate histological assessment. This is particularly crucial for intact polypectomy specimens. Ideally, these should be placed immediately into formalin after endoscopic procurement. After adequate fixation smaller polyps may be bisected through the stalk, as for all benign colorectal polyps. Larger polyps (>10 mm) should be trimmed to leave a central portion containing the intact stalk, which should be identified and processed separately from the peripheral fragments, all of which need to be processed for histology. It is recommended that, routinely, at least three sections be examined microscopically from the block or blocks containing

the stalk, and more if stage pT1 CRC is diagnosed and any of the relevant features under evaluation are equivocal. The base margin of larger, sessile or semipedunculated lesions can be painted, but this is probably unnecessary for stalked polyps, the resection margin of which is usually obvious microscopically.

Criteria for the biopsy diagnosis of adenocarcinoma

A problem encountered routinely by pathologists while reporting biopsy and polypectomy specimens derived from both screening and symptomatic endoscopy relates to the minimum morphological criteria required to confirm a diagnosis of epithelial malignancy. According to the WHO definition, and as accepted in European guidelines, adenocarcinoma in the lower gastrointestinal tract requires invasion beyond the muscularis mucosae into submucosa (Figures 6, 7A & B).[42,43] A less stringent definition is used in other countries, including Japan and elsewhere in Asia, where the diagnosis is influenced more strongly by cytological features and the term 'intramucosal adenocarcinoma' is recognized.

The rationale for avoiding the term 'intramucosal adenocarcinoma', and carcinoma *in situ* (pTis), relates to the perceived lack of metastatic potential of glandular neoplasia confined to colorectal mucosa, purportedly related to a paucity of lymphatic channels in this location.[44] Use of these terms may put a patient at risk of overtreatment by surgical intervention, if this low risk of metastatic potential is not appreciated clinically. Thus, the term 'high-grade neoplasia/dysplasia' is recommended to encompass this level of intramucosal neoplasia.

It is important for the pathologist to adhere to strict morphological criteria in making a diagnosis of adenocarcinoma, although the tissue diagnosis assumes different levels of importance depending on the clinical scenario. For example, in a clinically obvious cancer, evident on endoscopy and/or imaging, confirmation of primary glandular neoplasia in a tumour that the endoscopist deems as unresectable

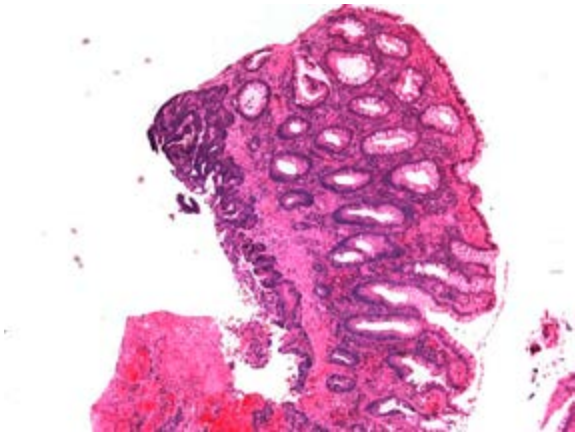


Figure 6. A biopsy from what was clinically and endoscopically rectal cancer. Is there definitive evidence of submucosal involvement? Although the neoplastic epithelium on the left appears to be in the submucosa, as it subtends non-neoplastic mucosa, this could represent unusual or tangential sectioning of a superficial adenomatous component. The difficulties with confirming submucosal involvement are clear. Nevertheless, the biopsy provides good evidence for primary glandular neoplasia.

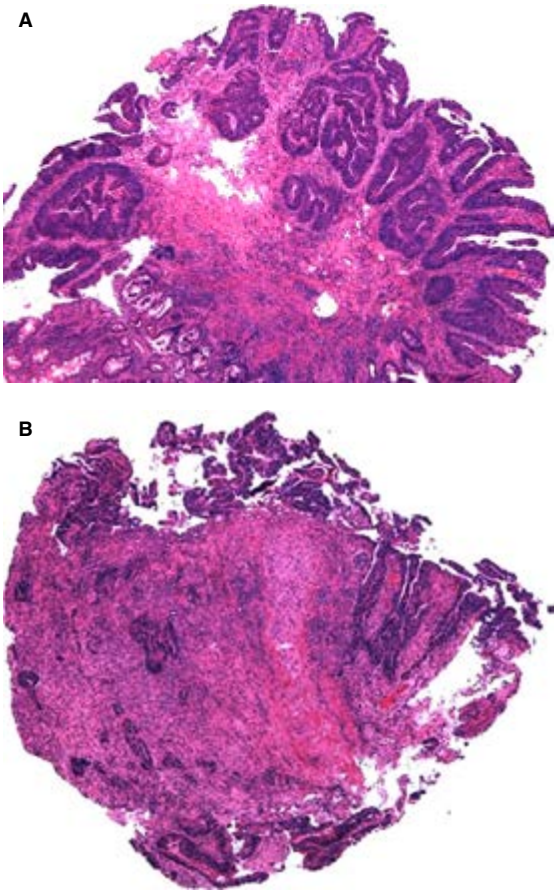


Figure 7. A, This is one biopsy from a lesion endoscopically and radiologically a cancer. There is high-grade dysplasia but no evidence of invasion into the submucosa. Adenocarcinoma cannot be confirmed on this biopsy. **B**, A further fragment from the same biopsy series as in **A**. Few would deny that the small irregular glands are infiltrative, but their presence in the submucosa is confirmed by the juxtaposition to a large arteriole (with endarteritis obliterans), a structure usually present only in the submucosa and not in the mucosa.

locally may be sufficient to proceed to surgery. Indeed, in national UK guidelines, we have championed this approach.[36] With an invasive tumour which has a superficial aspect composed largely of adenoma, multiple attempts at biopsy may fail to reveal the malignant component. If there is a large mass, especially in the colon, by both endoscopic and radiological analysis, and resection is warranted anyway, we see little point in repeated attempts at biopsy to confirm invasive adenocarcinoma, as long as the biopsies confirm primary colorectal glandular neoplasia (Figure 6).[36] Conversely, in biopsies from a polyp or polypoid lesion, a diagnosis of adenocarcinoma versus high-grade dysplasia assumes greater importance because of the associated differential risk of nodal metastasis and thus differing further management. However, the key management question remains of whether or not the lesion is endoscopically resectable. Management of adenomas is complete removal to prevent progression to cancer. Management of early ('polyp' or stage pT1) cancers is intact local excision, if possible, with careful consideration then of the pathological features in order to evaluate the need for further surgical intervention, to remove regional lymph nodes and stage fully.

The diagnosis of adenocarcinoma therefore is most important when a polypectomy specimen has been procured, the differential diagnosis typically being adenocarcinoma versus epithelial misplacement/pseudo-invasion. This differential diagnosis has been considered in detail above. Regarding endoscopic biopsy material derived from a clinically suspected tumour, the presence of neoplastic glands within a desmoplastic stroma, highly unusual in the setting of intramucosal neoplasia, is typically considered sufficient for a diagnosis of malignancy. Other less commonly observed features considered helpful for the demonstration of submucosal invasion include proximity of neoplastic glands to submucosal structures, such as thick-walled blood vessels, adipose tissue or nerves (Figure 7). In equivocal cases, a diagnosis of 'high-grade dysplasia' or 'suspicious of adenocarcinoma' may be more appropriate with subsequent correlation with endoscopic and radiological impressions at a multidisciplinary team meeting discussion. In many cases, such a diagnosis is sufficient to proceed to surgical resection.

Serrated pathology

The diagnosis and classification of colorectal serrated polyps, other than hyperplastic polyps, is a problem which is not peculiar to BCS pathology. Indeed, serrated pathology is discussed in much more detail in another review paper in this annual Review issue.[45] Guidelines addressing their reporting and management also form the basis of shortly-to-be-published British Society of Gastroenterology (BSG) guidelines.[46] Nevertheless, we will address some issues of serrated pathology pertinent to BCS.

One particular controversy is the classification of these lesions. In the United States and in WHO classifications, the term 'sessile serrated polyp/adenoma' is used for the increasingly recognized, predominantly right-sided serrated lesions. In the UK, we struggle with this classification because we believe that the term 'adenoma' should be restricted to lesions showing convincing morphological evidence of dysplasia and these lesions (usually) do not. Further, these lesions are certainly not always polypoid. Thus, in the UK and in other parts of Europe, the term 'sessile serrated lesion' is preferred. Within and without BCS, sessile serrated lesions are the second most commonly diagnosed serrated polyps after hyperplastic polyps. As they are typically sessile and non-ulcerated, they would not be expected to be enriched within FOB-based screening programmes, in comparison to large polypoid adenomas.

In national BCS programmes, endoscopists are exhorted to remove all polyps and, indeed, are judged, through rigorous colonoscopy accreditation processes, on their adenomatous polyp yield. We recognize that sessile serrated lesions are more difficult to identify at endoscopy because of their sessile nature, their proximal site and the frequent attachment of mucus plugs, obscuring the underlying lesion from the view of the unwary colonoscopist, and that adjunctive endoscopic methods, such as chromoendoscopy and narrow band imaging, enhance their detection. This underpins why such serrated lesions are more likely to be detected, at a higher rate, within rather than outside screening programmes.

Aside from diagnostic criteria and standardization of terminology, the other key question of some current controversy is the need for, and frequency of, follow-up surveillance colonoscopy after the detection of such pathology. Follow-up intervals for surveillance endoscopy after the diagnosis of adenoma(s) are well established, relating to the size and number of adenomas detected and corresponding risk stratification.[47] There is little evidence to allow us to address this question in relation to serrated polyps currently, although expert consensus opinion has been published, suggesting surveillance colonoscopy intervals of 1–5 years, depending on number, size, location

and histological type of serrated polyps diagnosed.[48] It is likely that BCS will generate useful data on significant numbers of serrated polyps and contribute to this evidence base, upon which future recommendations can be refined.

The effectiveness of screening

Pathology is just one aspect of a screening programme and must be considered in context. It is important to distinguish individual screening from organized or population screening. Many countries practise methods of early detection of CRC in individuals, often self-initiated or through private healthcare, but such activity can put pressure on endoscopy resources, especially if such individuals are at low risk and therefore screening is inappropriate.[49] Using available resources more efficiently may allow a programme to meet the demands of population screening.[50]

Population screening necessitates carefully developed protocols for the selection, identification and education of the target population, the issue of invitations, the performance of the screening test, and the management of those individuals positive for the screening test by follow-up investigation for diagnosis and treatment as appropriate. All aspects of the programme need to be organized and coordinated meticulously. The success of this will be reflected in programme effectiveness. Programme participation is an obvious but key factor in screening effectiveness. Participation in screening is determined by coverage, screening invitations being issued to the target population, and uptake, acceptance of the invitation by the target population. A huge range of reported uptake rates between different population-based programmes, from 17.2 to 90.1%, is a major reason for variable effectiveness of screening programmes.[51–53] An uptake of 45% is generally considered acceptable, although programmes should aim for at least 65%.[51–53] Discussion of reasons for uptake variation is beyond the scope of this review.

It is imperative that organized screening programmes include appropriate quality assurance structures addressing all aspects of screening, including pathology. A European group has recently produced a quality assurance document which, it is hoped, will be adopted across Europe and beyond, promoting collaboration and standardization in relation to CRC screening.[54]

Quality assurance of pathology is of paramount importance to screening programmes, as management depends upon diagnostic accuracy. As discussed above, pathology will typically confirm a diagnosis of adenocarcinoma in biopsies from endoscopically suspicious lesions, will diagnose and largely determine the need for surgical resection in stage pT1 cancers and, for those individuals found to have adenomas on colonoscopy but no cancer, will determine subsequent surveillance colonoscopy intervals. The latter is important to note, as such individuals constitute the vast majority of those undergoing colonoscopy after a positive FOB test.

The European guidelines for quality assurance of CRC screening include a comprehensive section relating to pathology, with 23 recommendations addressing a wide range of issues relevant to clinical practise.[43] These include: adoption of the Vienna two-tier classification for grading colorectal neoplasia; acceptance of the WHO definition of colorectal adenocarcinoma and WHO classification of adenomas; guidance on sizing polyps; guidance on substaging pT1 CRCs; use of proforma reporting both for biopsy and resection specimens; and specific standards relating to measurable outcomes from reporting of adenomas (for example, the proportion reported with high-grade dysplasia) and CRC resections (TNM stage distribution, lymph node harvests, and rates of circumferential margin involvement, peritoneal invasion and extramural venous invasion). Some of these standards have been adopted from the Royal College of Pathologists (UK) Dataset for Colorectal Cancer reporting.[36] It is hoped that setting such standards will focus attention upon key practise areas and lead to improvement in pathology practise in BCS and, in turn, within symptomatic reporting practise, through dissemination of education. Indeed, evidence of a notable improvement in overall quality of colorectal cancer reporting has already been seen as a result of the introduction of BCS, and its quality assurance procedures, in the UK. Further, external quality assurance and regular audit of individual practise and that of departments and whole programmes continue to be required to identify areas of concern.

Pathologists reporting in BCS programmes should therefore derive, or be provided with, their own reporting data and these should be evaluated individually and departmentally as part of the associated quality assurance programme. In addition to parameters pertaining to recognized quality standards, data pertaining to other parameters, such as proportions of tubular and non-tubular (tubulovillous or villous) adenomas, can provide a useful comparison with peers and can lead to equilibration of standards within departments, regionally and nationally. Multi-

header microscope sessions discussing such issues and sharing educational cases may be helpful and lead to quality improvement within departments. Such sessions also facilitate double reporting of cases, where appropriate.

Data relating to the impact of a screening programme on mortality reduction are not available until several rounds of screening have been completed. In advance of this, analysis of pathology stage data relating to detected cancers provides the most useful surrogate indicator of the success of a CRC screening programme. Accepting the limitations of such data relating to lead time and length bias, demonstration of a significant shift to earlier stage disease among screen-detected cancers, compared to symptomatic cancers, can be taken as evidence of BCS programme success.

References

1. Wilson JMG, Jungner G. *Principles and practice of screening for disease*. Geneva: WHO, 1968.
2. Lockhart-Mummery JP, Dukes C. The pre-cancerous changes in the rectum and colon. *Surg. Gynecol. Obstet.* 1928; **46**; 591–596.
3. Muto T, Bussey HJ, Morson BC. The evolution of cancer of the colon and rectum. *Cancer* 1975; **36**; 2251–2270.
4. Winawer SJ, Fletcher RH, Miller L *et al.* Colorectal cancer screening: clinical guidelines and rationale. *Gastroenterology* 1997; **112**; 594–642.
5. Greegor DH. Diagnosis of large-bowel cancer in the asymptomatic patient. *JAMA* 1967; **201**; 943–945.
6. Winawer SJ, Stewart ET, Zauber AG *et al.* A comparison of colonoscopy and double-contrast barium enema for surveillance after polypectomy. National Polyp Study Work Group. *N. Engl. J. Med.* 2000; **342**; 1766–1772.
7. Mandel JS, Bond JH, Church TR *et al.* Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *N. Engl. J. Med.* 1993; **328**; 1365–1371.
8. Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* 1996; **348**; 1467–1471.
9. Hardcastle JD, Chamberlain JO, Robinson MH *et al.* Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* 1996; **348**; 1472–1477.
10. Lansdorp-Vogelaar I, von Karsa L. Immunochemical FOBT: evidence for efficacy. In Senna N, Patnick J, von Karsa L eds. *European guidelines for quality assurance in colorectal cancer screening and diagnosis*. Luxembourg: IARC Press, 2010; 14–15.
11. Rex DK, Johnson DA, Anderson JC *et al.* American College of Gastroenterology guidelines for colorectal cancer screening. *Am. J. Gastroenterol.* 2009; **104**; 739–750.
12. Thiis-Evensen E, Hoff GS, Sauar J, Langmark F, Majak BM, Vatn MH. Population-based surveillance by colonoscopy: effect on the incidence of colorectal cancer. Telemark Polyp Study I. *Scand. J. Gastroenterol.* 1999; **34**; 414–420.
13. Gondal G, Grotmol T, Hofstad B, Bretthauer M, Eide TJ, Hoff G. The Norwegian Colorectal Cancer Prevention (NORCCAP) screening study: baseline findings and implementations for clinical work-up in age groups 50–64 years. *Scand. J. Gastroenterol.* 2003; **38**; 635–642.
14. Atkin WS, Edwards R, Kralj-Hans I *et al.* Once-only flexible sigmoidoscopy screening in prevention of colorectal cancer: a multicentre randomised controlled trial. *Lancet* 2010; **375**; 1624–1633.
15. Winawer SJ, Zauber AG, O'Brien MJ *et al.* The National Polyp Study. Design, methods, and characteristics of patients with newly diagnosed polyps. The National Polyp Study Workgroup. *Cancer* 1992; **70** (Suppl. S3); 1236–1245.
16. Baxter NN, Goldwasser MA, Paszat LF, Saskin R, Urbach DR, Rabeneck L. Association of colonoscopy and death from colorectal cancer. *Ann. Intern. Med.* 2009; **150**; 1–8.
17. Brenner H, Hoffmeister M, Arndt V, Stegmaier C, Altenhofen L, Haug U. Protection from right- and left-sided colorectal neoplasms after colonoscopy: population-based study. *J. Natl Cancer Inst.* 2010; **102**; 89–95.
18. Benson VS, Patnick J, Davies AK *et al.* Colorectal cancer screening: a comparison of 35 initiatives in 17 countries. *Int. J. Cancer* 2008; **122**; 1357–1367.
19. Klabunde C, Breen N, Meissner H, Subramanian S. Use of colonoscopy for colorectal cancer screening. *Cancer Epidemiol. Biomark. Prev.* 2005; **14**; 2279–2280.
20. Shepherd NA, Griggs RKL. Bowel cancer screening-generated diagnostic conundrum of the century: pseudo-invasion in sigmoid colonic polyps. *Mod. Pathol.* 2015; in press.
21. Muto T, Bussey HJR, Morson BC. Pseudocarcinomatous invasion in adenomatous polyps of the colon and rectum. *J. Clin. Pathol.* 1973; **26**; 25–31.
22. Hansen TP, Fenger C, Kronborg O. The expression of p53, Ki-67 and urokinase plasminogen activator receptor in colorectal adenomas with true invasion and pseudoinvasion. *APMIS* 1999; **107**; 689–694.
23. Mueller J, Mueller E, Arras E, Bethke B, Stolte M, Hofler H. Stromelysin-3 expression in early (pT1) carcinomas and pseudoinvasive lesions of the colorectum. *Virchows Arch.* 1997; **430**; 213–219.
24. Yantiss RK, Bosenberg MW, Antonioli DA, Odze RD. Utility of MMP-1, p53, E-cadherin, and collagen IV immunohistochemical stains in the differential diagnosis of adenomas with misplaced epithelium versus adenomas with invasive adenocarcinoma. *Am. J. Surg. Pathol.* 2002; **26**; 206–215.
25. Yantiss RK, Goldman H, Odze RD. Hyperplastic polyp with epithelial misplacement (inverted hyperplastic polyp): a clinicopathologic and immunohistochemical study of 19 cases. *Mod. Pathol.* 2001; **14**; 869–875.
26. Cooper HS, Deppisch LM, Kahn EI *et al.* Pathology of the malignant colorectal polyp. *Hum. Pathol.* 1998; **29**; 15–26.
27. Cooper HS. Surgical pathology of endoscopically removed malignant polyps of the colon and rectum. *Am. J. Surg. Pathol.* 1983; **7**; 613–623.
28. Hassan C, Zullo A, Risio M, Rossini FP, Morini S. Histologic risk factors and clinical outcome in colorectal malignant polyp: a pooled-data analysis. *Dis. Colon Rectum* 2005; **48**; 1588–1596.
29. Haggitt RC, Glotzbach RE, Soffer EE, Wruble LD. Prognostic factors in colorectal carcinomas arising in adenomas: implications for lesions removed by endoscopic polypectomy. *Gastroenterology* 1985; **89**; 328–336.
30. Kikuchi R, Takano M, Takagi K *et al.* Management of early invasive colorectal cancer. Risk of recurrence and clinical guidelines. *Dis. Colon Rectum* 1995; **38**; 1286–1295.

31. Ueno H, Mochizuki H, Hashiguchi Y *et al.* Risk factors for an adverse outcome in early invasive colorectal carcinoma. *Gastroenterology* 2004; **127**; 385–394.
32. Wang HS, Liang WY, Lin TC *et al.* Curative resection of T1 colorectal carcinoma: risk of lymph node metastasis and long-term prognosis. *Dis. Colon Rectum* 2005; **48**; 1182–1192.
33. Ishii M, Ota M, Saito S, Kinugasa Y, Akamoto S, Ito I. Lymphatic vessel invasion detected by monoclonal antibody D2-40 as a predictor of lymph node metastasis in T1 colorectal cancer. *Int. J. Colorectal Dis.* 2009; **24**; 1069–1074.
34. Egashira Y, Yoshida T, Hirata I *et al.* Analysis of pathological risk factors for lymph node metastasis of submucosal invasive colon cancer. *Mod. Pathol.* 2004; **17**; 503–511.
35. Bosch SL, Teerenstra S, de Wilt JH, Cunningham C, Nagtegaal ID. Predicting lymph node metastasis in pT1 colorectal cancer: a systematic review of risk factors providing rationale for therapy decisions. *Endoscopy* 2013; **45**; 827–834.
36. Loughrey MB, Quirke P, Shepherd NA. *Standards and datasets for reporting cancers: dataset for colorectal cancer*. 3rd ed. London: Royal College of Pathologists, 2014.
37. Ramirez M, Schierling S, Papaconstantinou HT, Thomas JS. Management of the malignant polyp. *Clin. Colon Rectal Surg.* 2008; **21**; 286–290.
38. Ali SM, Kalla R, Hendrickse M. Endoscopic mucosal resection of large colorectal polyps in non screening colonoscopies has a high recurrence rate. *Gut* 2013; **62**(Suppl. 1); A236.
39. Brooker JC, Saunders BP, Shah SG, Williams CB. Endoscopic resection of large sessile colonic polyps by specialist and non-specialist endoscopists. *Br. J. Surg.* 2002; **89**; 1020–1024.
40. Han KS, Sohn DK, Choi DH *et al.* Prolongation of the period between biopsy and EMR can influence the nonlifting sign in endoscopically resectable colorectal cancers. *Gastrointest. Endosc.* 2008; **67**; 97–102.
41. Kim EK, Han DS, Kim HS. A biopsy before the colorectal ESD – is it useful? *Gastrointest. Endosc.* 2012; **75**(Suppl. 4); AB407.
42. Hamilton SR, Bosman FT, Boffetta P *et al.* Carcinoma of the colon and rectum. In Bosman FT, Carneiro F, Hruban RH, Theise ND eds. *WHO classification of tumours of the digestive system*. 4th ed. Lyon: IARC Press, 2010.
43. Quirke P, Risio M, Lambert R, von Karsa L, Vieth M. Quality assurance in pathology in colorectal cancer screening and diagnosis – European recommendations. *Virchows Arch.* 2011; **458**; 1–19.
44. Compton C, Fenoglio-Preiser CM, Pettigrew N, Fielding LP. American Joint Committee on Cancer Prognostic Factors Consensus Conference: Colorectal Working Group. *Cancer* 2000; **88**; 1739–1757.
45. O'Brien MJ. Serrated pathology. *Histopathology* 2015; in press.
46. Bateman AC, Shepherd NA. Serrated lesions – nomenclature. British Society of Gastroenterology Serrated Lesion Working Group. 2014, in press.
47. Cairns SR, Scholefield JH, Steele RJ *et al.* Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* 2010; **59**; 666–689.
48. Rex DK, Ahnen DJ, Baron JA *et al.* Serrated lesions of the colorectum: review and recommendations from an expert panel. *Am. J. Gastroenterol.* 2012; **107**; 1315–1329.
49. Burnand B, Harris JK, Wietlisbach V, Froehlich F, Vader JP, Gonvers JJ. Use, appropriateness, and diagnostic yield of screening colonoscopy: an international observational study (EPAGE). *Gastrointest. Endosc.* 2006; **63**; 1018–1026.
50. Seeff LC, Manninen DL, Dong FB *et al.* Is there endoscopic capacity to provide colorectal cancer screening to the unscreened population in the United States? *Gastroenterology* 2004; **127**; 1661–1669.
51. Moss S, Ancelle-Park R, Brenner H. Chapter 3. Evaluation and interpretation of screening outcomes. In Segnan N, Patnick J, von Karsa L eds. *European guidelines for quality assurance in colorectal cancer screening and diagnosis*. Luxembourg: IARC Press, 2010; 71–102.
52. Faivre J, Arveux P, Milan C, Durand G, Lamour J, Bedenne L. Participation in mass screening for colorectal cancer: results of screening and rescreening from the Burgundy study. *Eur. J. Cancer Prev.* 1991; **1**; 49–55.
53. Zorzi M, Falcini F, Fedato C *et al.* Screening for colorectal cancer in Italy: 2006 survey. *Epidemiol. Prev.* 2008; **32**(Suppl. 1); 55–68.
54. Segnan N, Patnick J, von Karsa L eds. *European guidelines for quality assurance in colorectal cancer screening and diagnosis*. Luxembourg: IARC Press, 2010.

L 41

Gleason Grading
An Update

Murali Varma
Cardiff, UK
wptmv@cf.ac.uk

Ghent 2015

Why grade tumours?

- **Groups of patients**
  - **Clinical trials**
    - Groups have to comparable
  - **Survival analysis**
    - Surgeons, Areas (eg. Wales vs. England)
- **Individual patient**
  - Prognosis
  - Management

Grading tumours

- **Groups of patients**
  - Borderline grades cancel each other
  - Inter-observer reproducibility less important
  - Fewer tiers the better?
- **Individual patient**
  - Arbitrary lines in continuum
  - More tiers the better?
  - Inter-observer reproducibility critical

Outline of lecture

- **A historical perspective**
- **ISUP 2005:** What changes? Why changes?
- **ISUP 2014:** More changes/clarifications
- **Problems and controversies: my approach**
- **A radical new proposal for grade grouping**
  - ISUP 2014: proposed by Jonathan Epstein

Classical Gleason system

- Prospective clinical trial
- 1032 patients, 1960-75
- Pre-PSA, most (86%) advanced cancer
- Specimen type
  - TURBT, open prostatectomy, 14 gauge needle bx
- Pre-immunohistochemistry
- Before variants described

Development of Gleason system
Critical role of statisticians

- Team of statisticians from National Institutes of Health and National Cancer Institutes
- Headed by John C Bailar III
- Condensed Gleason's original 9 patterns into 5 grades
- Developed idea of a "score" created by adding most and second most prevalent grades
  - Based on analysis of survival data
  - Predominant (1-5) + second prevalent (1-5) + clinical stage (1-5)
    - Ranged from 1 + 1 + 1 = 3 to 5 + 5 + 5 = 15
    - Later clinical stage excluded from histological score

Gleason score: *Not by the Worst Grade*

- Minor component (eg. 6%) lower grade improves prognosis?
- **Rationale:**
  - volume of high-grade important?
  - marker of less aggressive behaviour?
    - 4 + 3: less aggressive 4 with residual 3
    - 4 + 4: more aggressive 4 with no residual 3
  - 4 + 4 and 4 + 3: separate diseases?
    - de-novo pattern 4 vs. transformation of 3

Classical Gleason system: *Principles*

- Low-medium power assessment
- Based only on architecture
- Not by worst pattern
- Grade included in score only if at least 5%
- Rules same for all specimen types
- Tertiary grade not included in score

Why was Gleason system adopted?

- Simple
- Quick
- Low/medium power evaluation
- Gleason's diagram of patterns easy to follow
- Based on follow-up data

Classical Gleason system: *Obsolete?*

- **Different patients**
  - Pre-PSA, advanced cancer (86%)
- **Different specimens**
  - TURPs, open prostatectomies, thick needle bx
    - No 18 gauge needle biopsies
- **Different lesions**
  - Pre-immunohistochemistry
    - GP 1 cancer: adenosis?
    - GP 5 with comedonecrosis: intraductal cancer?

International Society of Urological Pathology (ISUP) consensus conferences

- **2005:** USCAP meeting in San Antonio
  - 52 urological pathologists
  - Consensus defined as 2/3rd agreement
  - Several modifications of Gleason suggested
  - Am J Surg Pathol. 2005;29:1228-1242
- **2014:** Specially convened meeting in Chicago
  - 66 expert pathologists (and 17 clinicians) from 17 countries.
  - Results pending publication

ISUP 2005 modifications

- Use of Gleason scores 2-4
- Grading cribriform cancer
- Definition of pattern 4
- Grading variants
- Grading limited (<5%) secondary pattern
- Reporting tertiary pattern

Gleason Score 2-4

- **Needle biopsy**
  - Pattern 1: never
  - Pattern 2: very rarely
- **TURPs**
  - Uncommon
  - Prognosis same as Gleason 5-6
- **Radicals**
  - Occasionally
  - Small anterior transition zone tumours

Rationale for Gleason score 2-4 changes

- Gleason 2-4 tumours generally small anterior tumours
- Not sampled by needle biopsy
- Most (? all) Gleason's 1 + 1 = 2 cancers were adenosis

Cribriform prostate cancer

- **ISUP 2005**
  - **Almost all pattern 4 (GP 5 if comedonecrosis +)**
    - Many of Gleason's cribriform pattern 3 were high-grade PIN or intraductal cancer
    - Criteria for cribriform pattern 3 vs. 4 arbitrary and poorly reproducible (size, outline etc)
    - Cribriform pattern 3 generally associated with usual pattern 4
- **ISUP 2014**
  - **All cribriform cancer high-grade (at least GP 4)**

Pattern 4

- Poorly formed glands included in pattern 4

Grading *variants*

- **Ductal carcinoma:** pattern 4 at least
  - If comedonecrosis present: pattern 5
  - **PIN-like ductal cancer:** pattern 3
- **Pseudohyperplastic cancer:** pattern 3
- **Mucinous carcinoma**
  - ISUP 2005: No consensus
  - ISUP 2014: Grade based on underlying growth pattern

Grading *variants* (2)

- **Small cell carcinoma**
  - Do not grade
- **Cancer with vacuoles**
  - Ignore vacuoles
- **Foamy gland cancer**
  - Ignore foamy cytoplasm
- **Cancer with glomerulations**
  - no consensus (ISUP 2005)
  - Gleason pattern 4 (ISUP 2014)

<5% *lower* grade

- No change from original Gleason system
- Ignore in all specimens

<5% *higher* grade

- **Needle biopsies**
  - Include as secondary grade
- **TURPs and Radicals**
  - No consensus

Tertiary pattern *(of higher grade)*

- **Needle biopsy**
  - Include as secondary grade
- **Radicals**
  - Up to 5%: comment as tertiary pattern
  - >5%: no consensus

Gleason score in needle bx Primary + Worst: *Rationale*

- **Significant sampling error in needle bx**
  - Small amount of high-grade tumour in bx may be associated with large amount of high-grade in prostate
- **Tertiary pattern not included in nomograms**
  - 3 + 4 = 7 with tertiary 5 would be treated as Gleason score 7 in nomograms

EXAMPLES *(ISUP 2005)*

Pattern			Needle Biopsy	Radical Prostatectomy
3	4	5		
97%	3%		3 + 4	No consensus
3%	97%		4 + 4	4 + 4
60%	38%	2%	3 + 5	3 + 4 (tertiary 5)
60%	33%	7%	3 + 5	No consensus

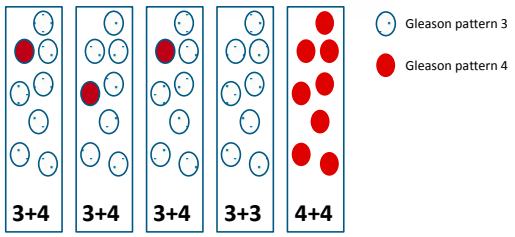
Epstein 2010 update *(J Urol 183:433-440, 2010)*

Pattern			Needle Biopsy	Radical Prostatectomy
3	4	5		
60%	33%	7%	3 + 5	3 + 5

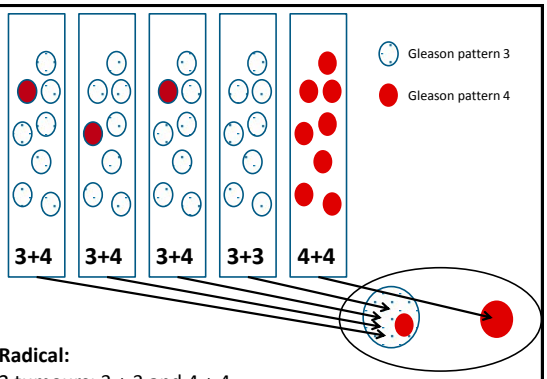
Needle biopsy with cores showing different grades

- **Cores submitted separately**
  - Score each core separately
- **Multiple cores in container**
  - No consensus
  - ? Score each core separately
  - ? Give score for container

Scenario 1

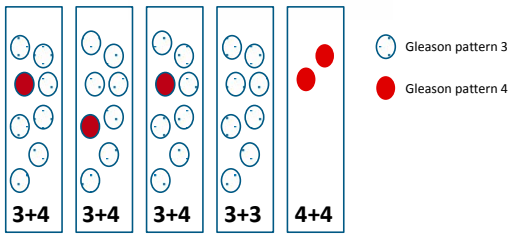


Gleason score:
Traditional: 3 + 4 = 7
ISUP 2005: 4 + 4 = 8

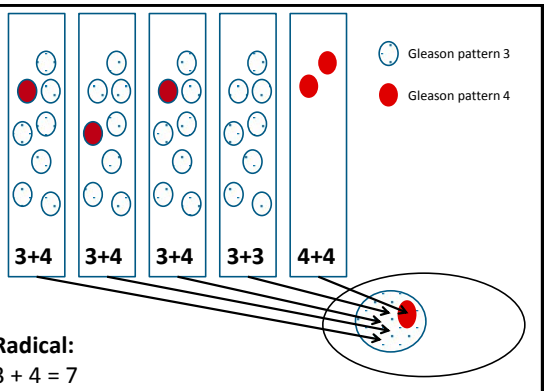


Radical:
2 tumours: 3 + 3 and 4 + 4
(ISUP 2005 correct as prognosis will be of 4 + 4 tumour)

Scenario 2



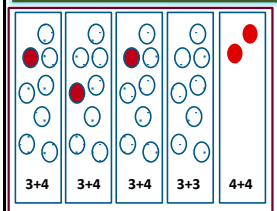
Gleason score:
Traditional: 3 + 4 = 7
ISUP 2005: 4 + 4 = 8



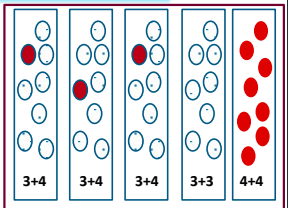
Radical:
3 + 4 = 7
(ISUP 2005 will over-grade in this scenario)

2 different scenarios

My approach

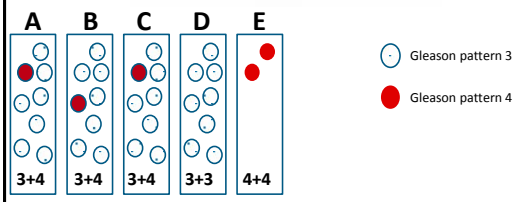


Overall Gleason score: 3 + 4 = 7



Overall Gleason score 3 + 4 = 7.
However, core E contains 7mm focus of only pattern 4 suggesting a separate 4 + 4 = 8 tumour

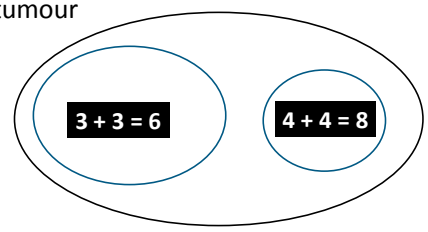
2 different scenarios *Another Approach*



Worst Gleason score 4 + 4 = 8 (core E). However, core E contains <1mm tumour so this score may not be representative

Radical with multiple tumours

- Assign separate score to each dominant tumour



- Prognosis will be of 4 + 4 = 8 rather than 3 + 4 = 7

2005 ISUP modified Gleason system: *Implications*

- **Gleason inflation**
  - Most common Gleason score 7
- Kuroiwa K et al. J Clin Path 2009; 62: 260-263
  - Upscoring in 35% cases
    - 90% of these due to highest core scoring
    - 10% due to revised 5% rule and tertiary rule

Intraductal prostatic carcinoma

- Almost always associated with invasive cancer
- Invasive adenocarcinoma growing within benign ducts
- Generally associated with high volume, high-grade, high-stage prostate cancer

Intraductal component with invasive *to grade or not to grade*

- **No (ISUP 2014):** Only invasive cancers graded
  - Recommend comment on invariable association with aggressive prostate cancer
- **Yes**
  - **Grading is for predicting prognosis**
    - Intraductal component predicts adverse outcome
  - **Intraductal may be morphologically indistinguishable from invasive**
    - If IDC not graded, then all high grade cancers would need immuno?

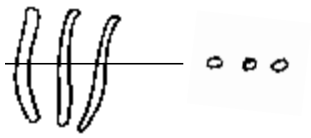
Grading: *Intrinsic problems*

- **Arbitrary lines**
  - Morphological continuum
  - Clinical continuum

What is glandular fusion?

- Not true fusion
- Increasing architectural complexity resulting to apparent fusion
  - Convoluted growth pattern with budding?
- Similar to atypical endometrial hyperplasia (back to back glands)

What is glandular fusion?



Gleason pattern 3

Gleason pattern 4

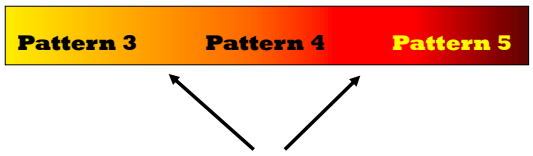
Borderline Gleason pattern 4

- How much complexity is required to diagnose pattern 4?
- Pattern 3 to 4 is a morphological continuum

Gleason patterns 3 – 5: *A clinical continuum*

Risk of EPE

1% 5% 10% 15% 20% 25% 30%



At what risk of EPE should the cut offs be?

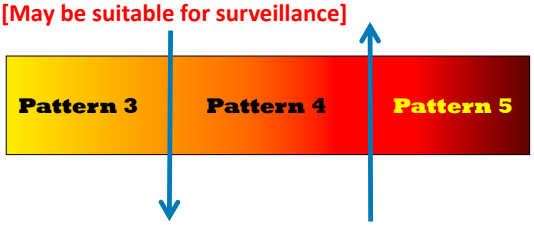
Borderline Gleason pattern 4 *My approach*

Use text comments such as

- 3 + 3 = 6 (foci bordering on pattern 4)
- 3 + 4 = 7 (borderline pattern 4)
- 3 + 4 = 7 (foci bordering on pattern 5)

Gleason score 3 + 4 = 7

3+4=7 (borderline pattern 4)
[May be suitable for surveillance]



3+4=7 (bordering on pattern 5)
[NOT suitable for surveillance]

Borderline terminology
Caveats

- Use sparingly
  - Only in truly indeterminate cases
- Audit use
- Ensure that urologist understands phrases
  - Use only after discussion with clinical team

Borderline Gleason pattern 4
Another approach

- **Report % pattern 4 in GS score 7 (ISUP 2014)**
  - Borderline pattern 4 generally <10% pattern 4
  - Rare to have large areas with borderline morphology
- **However these problems remain:**
  - 3 + 3 = 6 (bordering on pattern 4)
  - 3 + 4 = 7 (bordering on pattern 5)

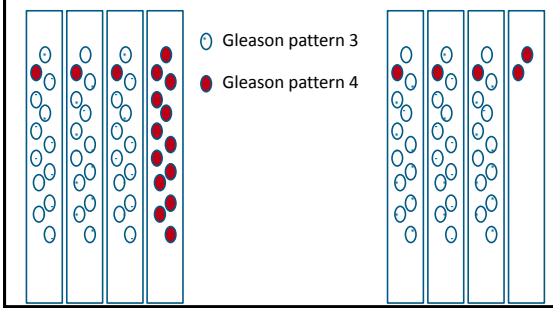
Grading: *Intrinsic problems*

- **Rigid rules**
  - 1 size fits all
  - No room for judgement

Reporting Gleason
2 scenarios, different requirements

- **Groups of patients**
  - Fewer tiers the better
  - Single simple score for entering in database
- **Individual patient**
  - More tiers better stratification
  - Clearly indicate where in morphological and clinical continuum

2 different scenarios, 1 Gleason score
ISUP 2005: both Gleason score 4 + 4 = 8



Grading :
Accuracy vs. reproducibility

- **Accuracy**
  - Impossible
  - No defined clinical cut-offs
    - Risk of EPE/mets/survival
  - Validation very difficult
- **Reproducibility**
  - Difficult

Reproducibility problems

- **Variable rules used**
  - Tertiary pattern in radicals (secondary?)
  - Grade intraductal component?
- **Vague definitions**
  - "Very few" glands in pattern 5
  - "Poorly formed glands"

Reproducibility problems (2)

- **Subjectivity of assessment**
  - Morphological continuum
    - Where should the lines be drawn?
  - Eyeball quantitation of 3 + 4 vs 4 + 3
    - May not be reliable in borderline cases
    - ISUP 2014: recommend reporting % pattern 4

Gleason grading: *Pitfalls*

- Small foci mis-interpreted as low grade
- Tailing of angulated pattern 3 glands mis-interpreted as pattern 5
- Tangential sectioning mimicking fusion
- Cribriform PIN mimicking cribriform 4
- Artificial overgrading with Rx effects
  - Gland atrophy mimics fusion

Gleason score: *Issues*

- **Gleason scores range from 2 to 10**
  - Needle bx: minimum score 6
  - Patients think GS 6 (out of 10) is intermediate grade
- **Risk groupings for Gleason score (variable)**
  - 2-4, 5-7, 8-10
  - 2-6, 7, 8-10
  - 2-6, 3+4, 4+3, 8-10

Grade grouping *Problems*

- **2-4, 5-7, 8-10**
  - Outcome similar for 2-6
  - 7 significantly worse than 6
- **2-6, 3+4, 4+3, 8-10**
  - 4+3=7 worse than 3+4=7 but both lumped as 7
- **2-6, 3+4, 4+3, 8-10**
  - Gleason 8 significantly better than 9-10

"ISUP/Epstein" grade groupings

I:	2 - 6
II:	3 + 4 = 7
III:	4 + 3 = 7
IV:	8
V:	9-10

Advantages
- 6 is in lowest group
- 3+4 and 4+3 separated
- No need to distinguish between 4+5, 5+4, 5+5

"ISUP/Epstein" grade groupings
Problems: 3+4 vs 4+3

- **Sampling error of biopsy**
  - 4 + 3 in bx: often 3 + 4 in radical
- **Morphological and clinical continuum**
  - 55% pattern 4 not significantly worse than 45%
    - Recommend reporting % pattern 4

Summary

- **Grade is a morphological and clinical continuum**
- **Population based analysis and individual patient management have different requirements from grading systems**
  - Reproducibility of borderline grades may be critical for patients
- **Academic and clinical practices have different needs**
  - Less tiers good for academic but not for clinical

My approach to grading
Personalised medicine

- Primarily for patient management
- Pathologist must use judgement to determine where the biopsy lies in the morphological and clinical spectrum
- Pathologist must clearly communicate this information to the urologist

My approach to grading
Advantages

- **Complements rather than replaces standard guidelines**
  - Better communication rather than different interpretation
- **Suitable for academic and clinical purposes**
- **3 + 4 = 7 (borderline pattern 4)**
  - Research: 3 + 4 = 7
  - Clinical: consider active surveillance

My approach to grading
Advantages

- Better tumour stratification
- Better informed urologist
- Better informed patient
- Less stressed pathologist

References

1. Phillips JL, Sinha AA. Patterns, Art, and Context: Donald Floyd Gleason and the Development of the Gleason Grading System. Urology 2009;74:497–504.
2. Epstein JI, Allsbrook WC,Jr, Amin MB et al. The 2005 ISUP consensus conference on Gleason grading of prostatic carcinoma. *Am J Surg Pathol* 2005;29:1228-1242.
3. Pierorazio PM, Walsh PC, Partin AW et al. Prognostic Gleason grade grouping: Data based on the modified Gleason scoring system. *BJU Int* 2013;111:753-760.
4. Epstein JI, Zelefsky MJ, Sjoberg DD, et al. A contemporary prostate cancer grading system: A validated alternative to Gleason score. *Eur Urol* 2015; Jul 9. pii: S0302-2838(15)00557-6 [Epub ahead of print].

INVITED LECTURES

L 42

PATHOLOGY NETWORKS AND DATABASES FOR SOFT TISSUE TUMOURS

Jean-Michel Coindre – Institut Bergonié – Bordeaux – France

Sarcomas are rare and heterogeneous with many subtypes explaining the high level of diagnostic difficulty with frequent important therapeutic consequences. In 2009, a national network of pathologists has been set up in France with the main objective to perform a systematic histological review of every new sarcoma, gastro-intestinal stromal tumour and desmoid tumour. This network is composed of 3 coordinating centers with 17 reference centers as well as 15 competence centers. Every center follows the same rules regarding cases to be reviewed, use of immunohistochemistry and molecular tests, content of pathology report, management of paraffin blocks and frozen tissue, and traceability of cases. Results of the first five years showed that the network reviewed about 90% of expected cases with a discrepancy rate of about 30% of discordances for second opinion cases and 10% for systematic reviews. This network allowed storage of a paraffin block in the network centers for about 2500 cases and of frozen tissue for 1000 cases out of 4500 cases reviewed per year.

Data are stored in real-time in a national database shared via Internet (https://rreps.sarcomabcb.org) and associated with clinical information collected by NetSarc (https://netsarc.sarcomabcb.org), the clinical network managing patient treatment and follow-up. Currently, this database contents data from 45000 patients and will be soon merge with the European database on sarcoma and GIST (conticabase : https://conticabase.sarcomabcb.org and conticaGist : https://conticagist.sarcoma.bcb.org) containing 18000 patients in the same warehouse (https://sarcomabcb.org). These databases represent a unique tool for research on mesenchymal tumours. We now have the project to integrate heterogeneous data, such as genomic, expression profiling and NGS data with the phenotypic data of the sarcomaBCB in the open source platforms I2B2 and Transmart in order to make available to the clinicians a tool for the best selection of targeted treatment for homogeneous groups of patients and to the researchers a tool for their research projects.

L 43

FLEMISH COLLABORATIVE GLOMERULONEPHRITIS GROUP (FCGG): FOUNDATION OF A RENAL PATHOLOGY REGISTRY

Wim Laurens – Nephrologist – AZ Nikolaas

At the end of 2014, the **Flemish Collaborative Glomerulonephritis Group** (FCGG) was founded on the initiative of several Flemish nephrologists to enhance interhospital collaboration for patients with glomerulonephritis.

The FCGG aims to improve the diagnosis and the treatment of patients with glomerulonephritis. Additionally, the FCGN wants to enhance interuniversitary and interhospital collaboration for purposes of clinical research and education.

Since the beginning of 2015, (renal) pathologists have joined the steering committee of the group, which operates under the wings of the NVBN (Nederlandstalige Belgische Vereniging voor Nefrologie=Dutch speaking part of the Dutch-speaking Belgian Society of Nephrology).

As a first and important initiative, the FCGG is setting up a **registry of all renal biopsies** that are performed in Flanders. The current estimate is that +- 1000 native kidney biopsies a year will be enrolled in the database. Input will come monthly from the different centres and will enclude:

- clinical data as proteinuria, renal function, blood pressure, hematuria… at the time of biopsy
- clinical diagnosis according to the ERA-EDTA classification of renal disease
- pathology diagnosis and coding

Output will be reported twice a year to all members of NBVN (during the spring and autumn meeting). Also, data can be requested for research or other purposes from the Steering Committee of the registry.

Importantly, there is no aim to centralize reporting or archiving of biopsies. Neither need patients be referred to specific centres. The registry merely aims to provide a framework for evidence-based reporting, intercentre collaboration and eventually clinical research based on knowledge of incidence and presentation of glomerulonephritis. Although the primary aim is focussed on glomerulonephritis, for reasons of completeness and clarity all biopsies from native kidneys will be enrolled in the system.

The start of the registry is planned for beginning 2016. Three University Centres (UZLeuven, UZ Gent and UZ Antwerpen) will be charged with the practical task of entry of data into the registry.

INVITED LECTURES

To standardize the input of data, efforts are under way to harmonize the trajectory between the biopsy procedure itself and the final pathology report.

In the current talk, we will present the necessary steps to take:

- uniform informed consent for renal biopsy

- uniform Renal Research Biobank form

- uniform application form for renal biopsy

- uniform preservation and transport of renal biopsy

- uniform pathology reporting and coding (with outline of classification to be used)

We thank all the Flemish Nephrology Departments for their continuous participation in and collaboration with the FCGG.

ORAL PRESENTATIONS

ORAL PRESENTATIONS

O 01

SPECIMEN INTEGRITY AND IMPROVED MARGIN ANALYSIS IN ENDOSCOPIC SUBMUCOSAL DISSECTION MAY AVOID SURGERY

Dessain A. (1), Snauwaert C. (1), Piessevaux H. (1), Jouret-Mourin A. (1) /
[1] Cliniques Universitaires Saint-Luc, Brussels

Introduction:

Endoscopic submucosal dissection (ESD) allows en-bloc resection of superficial gastro-intestinal tumors, thereby providing qualitatively exploitable samples. This contrasts with standard endoscopic mucosal resection (EMR), in which fragmentation precludes adequate pathological evaluation. Diagnostic accuracy of tumor staging and sub-staging in ESD specimens is crucial since it may prevent resective surgery. Furthermore, we developed an original macroscopic management based on perpendicular section of the lateral margins. As opposed to conventional parallel sectioning, we suggest that perpendicular sectioning may decrease false positive lateral margins. The present study explores how specimen integrity and improved margin analysis help avoid unnecessary surgery in a large series of colorectal (CR) ESD patients.

Aim:

The present study explores how specimen integrity and improved margin analysis help avoid unnecessary surgery in a large series of colorectal (CR) ESD patients.

Methods:

Between 2006 and 2014, 192 CR ESD were performed at St-Luc University Hospital, Brussels, Belgium. Lesion location, TNM classification, grade, surface area, and width and depth of sub-mucosal invasion were prospectively recorded. Traditional parallel sectioning was used from 2006 to 2009 (n=55). Perpendicular sectioning was used from 2010 to 2014 (n=127). The remaining ten specimens were fragmented and non-orientable; margins were therefore not analyzed. The number of positive margins, as well as patient outcomes, including local recurrence, need for surgery and disease-free survival, were studied according to section method.

Results:

We found low- and high-grade dysplasia and adenocarcinoma in 51 (26.6 %), 74 (38.5 %) and 67 (34.9 %) patients, respectively. Twenty-two (11.5 %) lesions were pT1, among which, 16 (72.7 %) were pT1sm1 and 6 (27.2%) were pT1sm2/3. Subsequent surgery was performed in 6/16 (37.5 %) pT1sm1 and all (6/6) pT1sm2/3 (p, 0.009). No samples showed residual carcinoma (pT0 N0) post-surgery. In the remaining 10/16 pT1sm1 (62.5 %), quarterly endoscopic follow-up for up to one year did not show recurrence.

Concerning margin analysis, 29/55 (52.7 %) margins were positive using parallel sectioning, among which 5 cases harbored high-grade dysplasia and 1 carcinoma. By contrast, perpendicularly cut margins were only positive in 38/127 (29.9 %; p, 0.003). Two had high-grade dysplasia and none had carcinoma. No residual lesion was found in patients who subsequently underwent surgery (n=8). Furthermore, no local recurrence was detected during long-term follow-up of up to six years, which suggests that positive margins should be regarded as false positives.

CONCLUSIONS:

In this large series of patients with CR lesions firstly managed by ESD, specimen integrity, i.e., one whole and non-fragmented piece, allowed for efficient orientation, diagnosis and therapy. Particularly, resective surgery could be significantly avoided in 62.5% of the pT1sm1 subgroup of patients. In addition, the macroscopic handling method based on perpendicular sectioning of lateral margins appears to significantly decrease false positive rates in CR ESD samples compared to conventional parallel sectioning. Altogether, specimen integrity and improved margin analysis complementarily improve diagnostic accuracy, thereby limiting unnecessary surgery in patients with superficial CR carcinoma.

O 02

TUMOR ASSOCIATED ANTIGENS IN UTERINE CERVICAL CANCER, DYSPLASIA AND NORMAL CERVICAL EPITHELIUM.

Eerdekens A. (1), Simoens C. (1), Schoolmeesters O. (1), Bogers J. (1) / [1] Laboratory for cell biology and histology, University of Antwerp, Antwerpen

Aim:

The current study was undertaken to investigate which tumor associated antigen (TAG) or combination of TAG's has the highest diagnostic accuracy to distinguish invasive uterine cervical squamous cell carcinoma (SCC),dysplasia and normal cervical epithelium.

Methods:

A retrospective study was performed, 99 cases including 34 SCC of the cervix, 33 normal cervical epithelium samples, 16 cervical intraepithelial neoplasia type I (CIN I) and 16 CIN III were selected. A preliminary systematic review was conducted to achieve a literature based list of TAG's with the highest diagnostic accuracy. Immunohistochemical assays of seven selected TAG's (VEGF, Survivin, p16, p14, PCNA, Cyclin D, Bcl-2) were performed on formalin-fixed paraffin-embedded cervical biopsy samples.

Results:

For each individual sample the percentage of stained cells, the location of staining (nucleus/cytoplasm/both) and the distribution of stained cells(basal layers of the epithelium/full epithelium) were determined. Sensitivity and specificity, stepwise backward, stepwise forward and single logistic regression were used to determine which TAG or combination of TAG's is most suitable to distinguish between SCC, dysplasia and normal cervical epithelium. It is clinically relevant to make a distinction between SCC and high grade dysplasia on one hand and low grade dysplasia and normal cervical epithelium on the other hand. Based on staining pattern and statistical analysis, p16 was found to be the best TAG to distinguish SCC and CIN III from CIN I and normal cervical epithelium (sensitivity 97.7%, specificity 87.2%).The diagnostic accuracy of the other TAG's was markedly less (p14: sensitivity 81.2%, specificity 4.65%, VEGF: sensitivity 100%, specificity 6.5%, Survivin: sensitivity 70.2%, specificity 17.8%, Bcl-2: sensitivity 51%, specificity 84.7%, PCNA: sensitivity 100%, specificity 17.8%, Cyclin D: sensitivity 56.7%, specificity 6.67%). No combination of TAG's was more suitable to distinguish SCC and CIN III from CIN I and normal cervical epithelium in comparison to the single use of p16. P16 was also found to be the best TAG to distinguish SCC from dysplasia and normal cervical epithelium (sensitivity 97%, specificity 67.8%) and to distinguish SCC and dysplasia from normal cervical epithelium (sensitivity 77.4%, specificity 87.5%).In these two models, the predictive values of the other TAG's were also less than those of p16 and no combination of TAG's was more suitable to make the abovementioned distinctions in comparison to the single use of p16.

Conclusions:

In this study it can be concluded that p16 has the highest diagnostic accuracy in making the distinction between SCC and high grade dysplasia versus low grade dysplasia and normal cervical epithelium. P16 was also found to be the best TAG to distinguish SCC from dysplasia and normal cervical epithelium and to distinguish SCC and dysplasia from normal cervical epithelium. Moreover, no combination of selected TAG's is superior to the single use of p16.

O 03

NEW INSIGHTS IN THE MOLECULAR PATHOGENESIS OF EPSTEIN-BARR VIRUS-POSITIVE AND -NEGATIVE POST-TRANSPLANT DIFFUSE LARGE B-CELL LYMPHOMA

Morscio J. (1), Finalet Ferreiro J. (1), Dierickx D. (1), Verhoef G. (1), Wlodarska I. (1), Tousseyn T. (1) / [1] UZ Leuven Gasthuisberg, Leuven,

Introduction:

Endoscopic submucosal dissection (ESD) allows en-bloc resection of superficial gastro-intestinal tumors, thereby providing qualitatively exploitable samples. This contrasts with standard endoscopic mucosal resection (EMR), in which fragmentation precludes adequate pathological evaluation. Diagnostic accuracy of tumor staging and sub-staging in ESD specimens is crucial since it may prevent resective surgery. Furthermore, we developed an original macroscopic management based on perpendicular section of the lateral margins. As opposed to conventional parallel sectioning, we suggest that perpendicular sectioning may decrease false positive lateral margins. The present study explores how specimen integrity and improved margin analysis help avoid unnecessary surgery in a large series of colorectal (CR) ESD patients.

Methods:

Between 2006 and 2014, 192 CR ESD were performed at St-Luc University Hospital, Brussels, Belgium. Lesion location, TNM classification, grade, surface area, and width and depth of sub-mucosal invasion were prospectively recorded. Traditional parallel sectioning was used from 2006 to 2009 (n=55). Perpendicular sectioning was used from 2010 to 2014 (n=127). The remaining ten specimens were fragmented and non-orientable; margins were therefore not analyzed. The number of positive margins, as well as patient outcomes, including local recurrence, need for surgery and disease-free survival, were studied according to section method.

Results:

We found low- and high-grade dysplasia and adenocarcinoma in 51 (26.6 %), 74 (38.5 %) and 67 (34.9 %) patients, respectively. Twenty-two (11.5 %) lesions were pT1, among which, 16 (72.7 %) were pT1sm1 and 6 (27.2%) were pT1sm2/3. Subsequent surgery was performed in 6/16 (37.5 %) pT1sm1 and all (6/6) pT1sm2/3 (p, 0.009). No samples showed residual carcinoma (pT0 N0) post-surgery. In the remaining 10/16 pT1sm1 (62.5 %), quarterly endoscopic follow-up for up to one year did not show recurrence.

Concerning margin analysis, 29/55 (52.7 %) margins were positive using parallel sectioning, among which 5 cases harbored high-grade dysplasia and 1 carcinoma. By contrast, perpendicularly cut margins were only positive in 38/127 (29.9 %; p, 0.003). Two had high-grade dysplasia and none had carcinoma. No residual lesion was found in patients who subsequently underwent surgery (n=8). Furthermore, no local recurrence was detected during long-term follow-up of up to six years, which suggests that positive margins should be regarded as false positives.

Conclusions:

In this large series of patients with CR lesions firstly managed by ESD, specimen integrity, i.e., one whole and non-fragmented piece, allowed for efficient orientation, diagnosis and therapy. Particularly, resective surgery could be significantly avoided in 62.5% of the pT1sm1 subgroup of patients. In addition, the macroscopic handling method based on perpendicular sectioning of lateral margins appears to significantly decrease false positive rates in CR ESD samples compared to conventional parallel sectioning. Altogether, specimen integrity and improved margin analysis complementarily improve diagnostic accuracy, thereby limiting unnecessary surgery in patients with superficial CR carcinoma.

O 04

RADIOTHERAPY-INDUCED DAMAGE TO CANCER-ASSOCIATED FIBROBLASTS PROMOTES COLORECTAL CANCER PROGRESSION

Tommelein J. (1), Verset L. (2), Gremonprez F. (1), Boterberg T. (1), de Tullio P. (3), Demetter P. (2), Bracke M. (1), De Wever O. (1) / [1] UZ Gent, Gent, [2] ULB/Erasme Hospital, Brussels, [3] CHU de Liège Hôpital du Sart Tilman, Liège

Introduction:

Preoperative radiochemotherapy increases life expectancy in rectal cancer patients through improved locoregional control. Interestingly, those patients with a fibrotic locoregional response show a poorer disease free survival and overall survival compared to those patients with a fibro-inflammatory response. Fibrosis is mainly an accumulation of fibroblasts. Tumors are ecosystems consisting of cancer cells and ostensibly normal cells such as cancer-associated fibroblasts (CAFs), immune cells and endothelial cells.

Aim:

Our project aims to understand how CAFs react to radiotherapy (RT) and if this reaction is implicated in fibrosis and colorectal cancer (CRC) cell survival mechanisms.

Methods:

Cultures of colorectal CAFs were irradiated with therapeutic doses of 1.8 Gy (daily during 10 days). Transcriptomics and proteomics were performed. Irradiated (2 Gy) and non-irradiated CRC cultures were treated with the secretome of irradiated (10 x 1.8 Gy) or non-irradiated CAFs and were evaluated by phase/contrast microscopy, NMR-based metabolomics, MTS, cell count and western blot. An orthotopic mouse model for CRC was used by intra-caecal injection of CRC cells. Tissue samples from patients with locally advanced rectal cancer were taken at the moment of diagnosis and at the moment of surgery. Immunohistochemistry was performed for phospho-mTOR and α-SMA.

Results:

Transcriptome profiling revealed a unique mRNA profile mostly enriched for genes implicated in cell cycle and survival including a significant upregulation of Insulin-like Growth Factor-1 (IGF-1), a pro-survival factor. A complementary protein array confirmed higher secretion of IGF-1 by irradiated CAFs. Treatment of irradiated and non-irradiated CRC cultures with the secretome of irradiated CAFs stimulates the IGF-1 receptor (Y1135/1136)/AKT (S473) survival pathway causing increased cell spreading, lactate release, extracellular acidification, metabolic activity and growth. These effects are mimicked by recombinant IGF-1 and are not observed with secretomes coming from non-irradiated CAFs.

Intra-caecal injection of CRC cells in a mouse model demonstrates tumors that are highly proliferative, show a CAF reaction and invade into the normal adjacent host tissue. In a next stage the image-guided small animal radiation research platform (SARRP) will be used, which allows the treatment of animal models of cancer more accurately and with planned protocols similar to those utilized in the clinic.

Quantification of phospho-mTOR (downstream IGF-1-R/AKT survival pathway) and α-SMA staining on tissue samples from patients is currently ongoing and will be compared between the samples before and after RT. α-SMA, a marker for CAFs, is associated with fibrotic response.

Conclusions:

Our data demonstrates a bystander-type phenomenon in which IGF-1 release by irradiated CAFs alters the phenotype of adjacent CRC cells. This research may reveal whether RT protocols combined with IGF-1/IGF-1 receptor targeting agents increase the efficacy of rectal cancer treatment.

O 05

SIGNIFICANCE OF PLASMA CELLS IN MELANOMA

Bosisio F. (1), van Baren N. (2), Mercier M. (2), Stas M. (1), Wouters J. (1), van den Oord J. (1) /[1] UZ Leuven Gasthuisberg, Leuven, [2] Ludwig Institute for Cancer Research and de Duve Institute

Introduction:

Despite the beneficial effects of humoral immunity in vivo and in vitro, several small studies have claimed that melanomas harboring sheets of plasma cells (PC) carry a poor prognosis.

Aim:

We aimed at characterize primary melanomas associated with plasma cells and to study the nature of these plasma cells. Moreover, we investigated on the origins of the melanoma-associated plasma cells. Finally, we studied the outcome of patients with primary melanomas associated with plasma cells.

Methods:

We revised 710 melanomas to correlate the presence of PC with histological prognostic markers. Immunohistochemistry for CD138, heavy and light chains was done in PM and LN. In 3 PM and in 9 LN with frozen material, VDJ-rearrangement was analyzed by Gene Scan Analysis. Survival analysis were perfomed on a group of 85 melanomas standardized for Breslow thickness (>2mm).

Results:

41 cases (5%) showed clusters/sheets of PC. PC-rich melanomas occurred at an older age and were thicker, more often ulcerated and more mitotically active ($p<0.05$). PC were polyclonal and often expressed IgA in addition to IgG. In LN, IgA+ PC were found both in the sinuses and subcapsular areas. The number of IgA+ PC was higher in involved than in non-involved LN. Analysis of VDJ-rearrangements showed the IgA to be oligoclonal. The Kaplan-Meier curves of survival showed that melanomas with clusters and sheets of plasma cells had a significantly worse survival compared to melanomas without plasma cells while, interestingly, melanomas with sparse plasma cells in the infiltrate had a better survival.

Conclusions:

Melanoma with plasma cells in the inflammatory micro-environment are rare (3.7%), occur at older age, and are associated with adverse histological markers of prognosis and with a worse survival. Melanoma-associated plasma cells are predominantly of the IgG and IgA isotypes; IgA producing plasma cells can be detected in the tumor-draining loco-regional lymph nodes. IgA oligoclonality suggests an antigen-driven response that facilitates melanoma progression by a hitherto unknown mechanism.

ORAL PRESENTATIONS

O 06

FIRST BELGIAN NATIONAL EXTERNAL QUALITY ASSESSMENT FOR SPECIAL STAINS IN HISTOPATHOLOGY

Ghislain V. (1) , Egele C. (2), Verbeke H. (1), Coucke W. (1), Michiels J. (2), Bellocq J. (2), Galant C. (3), Pauwels P. (3), Pétein M. (3), Van den Heule B. (3), Van Campenhout C. (1) / [1] «Quality of Medical Laboratories, Scientific Institute of Public Health, Brussels, Belgium [2] AFAQAP (Association Française d'Assurance Qualité en Anatomie Patholgique), Strasbourg, France, [3] Working group EQA, Commission of anatomic pathology, Brussels, Belgium

Introduction:

In 2014, the first national survey for special stains in histopathology was organized by the Belgian Scientific Institute of Public Health, in collaboration with the Commission of anatomic pathology. EQA programs have become imperative to give confidence in the quality of laboratory testing and to ensure delivery of consistent and accurate results that, ultimately, impact a patient diagnosis. They enable laboratories to evaluate their performance and methods compared with that of their peers or with the whole laboratory community. Participation is mandatory for Belgian licensed pathology laboratories and the goal of our programs is to be educational rather than to be repressive. This abstract provides an insight into the first (2014) and the second survey (2015) for special stains in histopathology.

Aim:

The two surveys aimed at assessing laboratory and method performance of the Belgian licensed pathology laboratories. General quality of the slides (sectioning and coverslipping), of the hematoxylin and eosin (HE) stain and of four special stains (PAS, reticulin, trichrome and Perls), as submitted by the participants, was evaluated. All laboratories were encouraged to process the samples within the laboratory's regular workload of patient specimens, using their routine methods.

Methods:

A panel of two formalin-fixed and paraffin-embedded tissue samples, liver and kidney, was provided to each participant, along with a request for sectioning and staining (HE, PAS and reticulin in 2014 and HE, PAS, reticulin, trichrome and Perls in 2015). Participants were surveyed for methodology and were instructed to return the stained slides within a two-week time frame. An expert panel of two pathologists evaluated the submitted slides for histologic technique using pre-established criteria. General quality of the slides and quality of each stain was assessed as optimal, good, borderline or poor. For each survey, an overall assessment score was generated which allowed us to compare all laboratories' performances. Since not all participants routinely perform all requested special stains, this overall assessment score was based upon slide quality and HE stain only. A general report including the encrypted results of all participants, individual comments, recommendations

and assessment marks for staining methods was published. Following the first survey and prior to the next, follow-up on poor performing laboratories was conducted by the program organizers.

Results:

Ninety-one laboratories participated in the 2014 survey of which 73% achieved an excellent overall score. Eighty-eight laboratories participated in the 2015 survey of which 80% achieved an excellent overall score. In 2014, the percentage of participants that achieved a satisfactory mark (optimal or good) for slide quality was 80%, for the HE stain 90%, for the PAS stain 76% and for the reticulin stain 81%. In 2015, the percentage of participants that achieved a satisfactory mark (optimal or good) for slide quality was 82%, for the HE stain 97%, for the PAS stain 82%, for the reticulin stain 76%, for the trichrome stain 72% and for the Perls stain 92%. A statistically significant difference between results obtained with manual vs. automated staining methods was observed.

Conclusions:

These surveys assessed the laboratory and method performance of the Belgian pathology laboratories currently licensed for routinely performing histopathological stains. The results suggest that the vast majority of the Belgian laboratories are able to provide good quality histopathological stains. Moreover, differences between staining methods were observed. By providing a general report with individual comments and follow-up on poor performances, the program aims at stimulating the participants to adopt proper practices for histopathological stains and hence improving their quality.

NOTES

e-POSTERS

e-POSTERS

e-Posters Morning Session:

e-POSTERS

e-Posters Afternoon Session:

P 01

THE PROGNOSTIC VALUE OF THE VEGFR-1 AND -2 COMBINATION IN ENDOTHELIAL CELLS OF COLORECTAL CANCER

D'Haene N. (1), Koopmansch C. (2), Allard J. (2), Hulet F. (3), Moles Lopez X. (2), Van Eycke Y. (2), Decaestecker C. (1), Salmon I. (1) / [1] Hôpital Erasme, Brussels, [2] CUB ULB Hopital Erasme, Brussels, [3] CHU Brugmann Site Victor Horta, Brussels

Introduction:

Research on tumour angiogenesis has mainly focused on vascular endothelial growth factor (VEGF) family and methods to block its actions. However, reports on the VEGF receptor (VEGFR) expression in tumor-associated endothelial cells (EC) are limited.

Aim:

We decided to evaluate immunohistochemical VEGFR1 and VEGFR2 expression in EC of colorectal cancer (CRC).

Methods:

EC VEGFR1 expression was quantitatively evaluated by computer-assisted microscopy in a retrospective series of 259 tumor tissues. Results were related to clinical variables.

Results:

The data show that the EC VEGFR1 and VEGFR2 expression are heterogenous. Using univariate analysis, high EC VEGFR1 expression is an independent negative prognostic factor in terms of metachronous metastasis ($p = 0.031$) and overall survival ($p = 0.004$). Low EC expression of VEGFR2 is an independent negative prognostic factor in terms of metachronous metastasis ($p = 0.043$). The combination of high VEGFR1 and low VEGFR2 is associated with improved metastasis-free survival ($p = 0.005$) and improved overall survival ($p = 0.04$). Using multivariate analysis, high EC VEGFR1 expression is still an independent negative prognostic factor associated with poor overall survival. The combination of high VEGFR1 and low VEGFR2 is an independent negative prognostic factor with regard to metastasis-free survival ($p = 0.007$) and overall survival ($p = 0.012$).

Conclusions:

This work illustrates the importance of studying distribution of VEGF members in EC of CRC. Interestingly, higher EC VEGFR1 expression and lower EC VEGFR2 expression appears as being involved in CRC progression, suggesting that targeting EC VEGFR1 could offer novel opportunities for CRC treatment.

P 02

EXPRESSION OF THE SMALL GTPASE RAB27B IS ASSOCIATED WITH STROMAL INFLAMMATION IN DUCTAL CARCINOMA IN SITU OF THE BREAST

Van Bockstal M. (1), De Wever O. (1), Denys H.(1), Braems G. (1), Van den Broecke R. (1), Cocquyt V. (1), Bracke M. (1), Libbrecht L. (1), Hendrix A. (1) / [1] UZ Gent, Gent

Introduction:

Ductal carcinoma in situ (DCIS) is regarded to be a non-obligate pre-invasive precursor of invasive ductal carcinoma. Some DCIS present with an inflammatory infiltrate in the periductal stroma, but the etiology of this stromal inflammatory response is currently unknown. Rab27B is a small GTPase that is involved in the release of exosomes, i.e. small intraluminal vesicles that are released upon fusion of multivesicular endosomes with the plasma membrane. Rab27B is upregulated in invasive ductal carcinoma, but its role in early breast cancer progression and the tumor immune microenvironment is still relatively unexplored.

Aim:

We aimed to investigate Rab27B expression in DCIS, as well as its relation with stromal inflammation.

Methods:

Investigations were performed on a cohort of 71 consecutive patients diagnosed with pure DCIS. The following histopathological features were assessed: DCIS architecture, nuclear grade, calcifications, extensive comedonecrosis, apocrine differentiation and peritumoral stromal inflammation. Immunohistochemistry for estrogen receptor (ER), progesterone receptor (PR) and Rab27B was performed on whole mount slides. HER2 amplification status was determined by dual-probe fluorescence in situ hybridization (FISH). Chi-square test and multivariate logistic regression analysis were performed to analyze which features were associated with stromal inflammation.

Results:

Thirty-five of 79 DCIS (49%) presented no or mild stromal inflammation, and 36 of 79 DCIS (51%) showed moderate to extensive inflammation in the periductal stroma. High nuclear grade ($p<0.001$), absence of intraductal calcifications ($p=0.042$), presence of extensive comedonecrosis ($p<0.001$), Rab27B overexpression ($p=0.048$), ER negativity ($p=0.007$) and PR negativity ($p<0.001$) were significantly associated with the presence of moderate to extensive stromal inflammation. DCIS architecture ($p=0.559$) and apocrine differentiation ($p=0.305$) were not associated with stromal inflammation. In multivariate analysis, high nuclear grade ($p=0.005$), HER2 amplification ($p=0.002$) and high Rab27B expression ($p=0.007$) were independently associated with the presence of moderate to extensive stromal inflammation in DCIS.

Conclusions:

We aimed to explore the underlying causes of stromal inflammation in DCIS. We have shown that DCIS lesions with moderate to extensive stromal inflammation present more often high nuclear grade, HER2 amplification and Rab27B overexpression. Rab27B is a secretory GTPase involved in vesicle trafficking and exocytosis. Secretory products of malignant epithelial cells might evoke a host inflammatory response in the tumor microenvironment. Further research is necessary to elucidate which secretory products might be responsible for this inflammatory response. Additional studies are required to investigate whether the presence of stromal inflammation and Rab27B overexpression are markers of poor prognosis in DCIS.

P 03

NFAT5 FORMS AGGREGATES IN NORMAL AND DUCHENNE MUSCULAR DYSTROPHY CULTURED MYOTUBES AFTER EXPOSURE TO CELL STRESSORS.

Herbelet S. (1), Beck I., Drebert Z., De Wever O. (1), De Bleecker J. / [1] UZ Gent, Gent

Introduction:

Like myodegeneration and fibrosis, Duchenne muscular dystrophy (DMD) is also characterized by chronic inflammation. In DMD, dystrophin malfunction causes Ca2+- and Na+-influx, which is deleterious to DNA. Nuclear factor of activated T-cells 5 (NFAT5) is upregulated and translocated to the nucleus in cells exposed to extracellular hyperosmolarity and the accompanying Na+-influx. In turn, NFAT5 induces the production of osmolytes which will restore cell homeostasis. NFAT5 is also activated by cytokines IL-1β and TNF- α.

Aim:

This study aims to understand the impact of cytokines IFN-γ, IL-1β and TNF- α and of increasing NaCl concentrations on NFAT5 expression and localization in normal and in Duchenne muscular dystrophy cultured myotubes.

Methods:

Myoblasts were grown in DMEM containing high glucose, additionally supplemented with 10% fetal calf serum, penicillin + streptomycine. After differentiation to myotubes, cells were exposed to cytokines IFN-γ, IL-1β and TNF- α or mixtures thereof. Increasing NaCl concentrations from 18mM to 100mM were added to the cultures. NFAT5 localisation and expression were studied by means of immunofluorescence (IF), Western-blotting and qPCR.

Results:

Our findings demonstrate that NFAT5 is mildly, yet consistently upregulated and is induced to form aggregates in normal and DMD myotubes after exposure to increasing hyperosmolar NaCl concentrations or cytokines IL-1β , IFN-γ and TNF-α or mixtures of these cytokines. These cytoplasmic aggregates accumulate in the perinuclear region of the cell. IF seems to colocalize NFAT5 aggregates with ubiquitin, whereas IF for markers of the Golgi apparatus and endoplasmic reticulum did not show any co-localization with the NFAT5 aggregates. IF staining of biopsies of patients suffering from DMD, dermatomyositis or polymyositis revealed NFAT5 aggregates accumulating around the myonuclei, resembling the picture obtained with our salt- or cytokine-induced myotubes. In chronic myositis as well as in muscular dystrophy with bystander inflammation, such as in DMD, abnormal NFAT5 proteomics may be a crucial component of interactions between inflammatory and degenerative pathomechanisms.

Conclusions:

The data help to better understand the pathology of muscular dystrophy as well as myositis.

P 04

GENOMIC PROFILING OF LUNG CANCER CYTOLOGICAL SAMPLES USING NEXT GENERATION SEQUENCING

D'Haene N. (1), Bienfait L. (1), Weynand B. (2), Le Mercier M. (1), De Nève N. (1), Blanchard O. (1), Salmon I. (1), Remmelink M. (1) / [1] Hôpital Erasme, Brussels, [2] CHU UCL Mont Godinne, Yvoir

Introduction:

NCCN guidelines recommend broader molecular profiling to identify rare actionable mutations for lung cancer patients. Next generation sequencing (NGS) has begun to supplant other technologies for gene panel sequencing. Cytological sample is sometimes the only material available for diagnosis of lung cancer. Therefore, molecular testing should be also validated on cytological samples, such as fine needle aspiration or pleural effusion.

Aim:

In the present study we evaluate the clinical applicability of targeted NGS on cytological lung cancer samples.

Methods:

The DNA of 93 formalin-fixed paraffin-embedded cell blocks was prospectively subjected to targeted NGS with the Ion Torrent AmpliSeq colon/lung cancer panel which interrogates 1850 hotspots in 22 cancer-related genes using the Ion Torrent Personal Genome Machine.

Results:

The set of 93 samples included 8 primary tumors and 85 metastatic lesions. Eighty-seven (94%) samples were adenocarcinomas. Eighty-five (91%) samples were successfully sequenced. The most frequent mutations were found in TP53 (49%) and KRAS (37.6%). Twenty potentially actionable mutations were identified (23.5 %), including 11 EGFR mutations, 2 PIK3CA mutations, 5 BRAF mutations, 1 PTEN mutation and 1 NRAS mutations.

Conclusions:

Overall, the AmpliSeq colon/lung cancer panel can be applied in daily practice for cell blocks. Moreover, it provides clinically relevant information for lung cancer patients.

P 05

DENSITOMETRY OF FEULGEN-STAINED HISTOLOGICAL SECTIONS HELPS TO DIAGNOSE PARTIAL HYDATIDIFORM MOLE.

Fastré E. , Khaing K (2), Guiot Y. (1), Van Der Smissen P. , Marbaix E. (1) / [1] Cliniques Universitaires Saint-Luc, Brussels, [2] University Hospital of Mangway, Myanmar

Introduction:

Partial hydatidiform mole (PHM) is associated with triploidy or tetraploidy that can be diagnosed by caryotype analysis. However, material is not available for genetic analysis in many instances of miscarriage and only formalin-fixed and paraffin-embedded (FFPE) material can be used to reach a diagnosis. Histopathological diagnosis of PHM is not easy because morphological features may overlap with hydropic miscarriages associated or not with other genetic anomalies and immunohistochemistry is of no help, leading to high interobserver and intraobserver variability. Patients with PHM are at risk for subsequent molar pregnancy or trophoblastic gestational disease, even if the risk is lower than for complete hydatidiform mole (CHM). We therefore think there is a need for a convenient tool to improve the diagnosis in routine pathological work.

Aim:

We wanted to develop a morphometric method that could help to diagnose PHM on FFPE miscarriage products with no available caryotype.

Methods:

74 FFPE samples of PHM, CHM, diploid miscarriages and miscarriages suspected to be PHM but without caryotype were used in the study. Sex chromosomes were labelled by fluorescent in situ hybridization in miscarriages with unknown caryotype. Histological sections were stained according to Feulgen or with 4',6-diamidino-2-phenylindole (DAPI). Optical density and area of nuclear profiles were measured on Feulgen-stained histological sections. Fluorescence level was measured on DAPI-stained histological sections.

Results:

Optical density, nuclear area, and their product were largely increased in stromal cells of chorionic villi in PHM compared to diploid cases and maternal endothelial cells. Optical density was also lower in decidual cells but nuclear profile area was larger, preventing to use these cells as diploid control. Feulgen densitometry also strongly supported triploidy in miscarriage products with 3 sex chromosomes compared to those with 2 sex chromosomes. DAPI fluorescence levels yielded similar results.

Conclusions:

Feulgen densitometry is an easy-to-use method that allows to confidently diagnose PHM on FFPE miscarriage products with no available caryotype.

P 06

CLINICAL APPLICATION OF TARGETED NEXT GENERATION SEQUENCING FOR GLIOBLASTOMA PATIENTS.

Trépant A (1), Le Mercier M. (1), Maris C; (1), De Nève N. (1), Blanchard O. (1), D'Haene N. (1), Salmon I. (1) / [1] Hôpital Erasme, Brussels

Introduction:

Glioblastomas (GBMs) are the most common malignant primary brain tumours in adults. These tumours are resistant to conventional treatment approaches including surgical resection, radiotherapy and chemotherapy. International efforts to catalogue mutations for multiple forms of cancer coupled with the successes of targeted agents in patients with molecularly defined tumors and improvements in genomic technology have generated enthusiasm for incorporating genomic profiling into clinical cancer practice. The development of tyrosine kinase inhibitor treatments has made it important to test cancer patients for clinically significant gene mutations that influence the benefit of treatment. Therefore, the number of biomarkers that will need to be assessed is expected to increase rapidly. Recently, next generation sequencing (NGS) has begun to supplant other technologies for gene panel sequencing. However, few studies have validated the use of targeted NGS for GBM patients. In the present study we evaluate the clinical applicability of targeted NGS for patients with GBM.

Methods:

DNA from 59 GBM samples was retrospectively subjected to targeted NGS with the Ampliseq Cancer Hotspot Panel, using the Ion Torrent Personal Genome Machine, which allowed us to analyze 2850 known cancer-related mutations in 50 genes and copy number variation for 24 genes. In addition, MGMT methylation status was evaluated by Methylation Specific PCR. All samples were successfully sequenced.

Results:

The most frequent mutations were found in TP53 (25%), and EGFR (17%). Potentially actionable mutations including 10 EGFR, 2 PIK3CA, 5 PTEN, 1 BRAF, 1 KRAS, 1 PDGFRA and 4 IDH1 mutations were identified in 22 patients (37%). Moreover, PDGFRA, MET and EGFR amplifications were detected for 11 (19%), 13 (22%) and 27 (46%) patients respectively

Conclusions:

Overall, the AmpliSeq Cancer Hotspot Panel can be applied in daily practice for GBM samples. Moreover, it can provide clinically relevant information for GBM patients.

P 07

CASE REPORT

POSTMORTEM DIAGNOSIS OF PHENYTOIN ASSOCIATED REACTIVE LYMPHADENOPATHY.

D'Hondt D. (1), Dendooven A. (1), Jacobs W. (1) / [1] Universitair Ziekenhuis Antwerpen, Antwerpen

Content:

Phenytoin is sometimes still used as an anticonvulsant in the treatment of epilepsy. It is known to cause side effects and hypersensitive reactions. We present a case report of a 27-year-old man who died under suspicious circumstances in his hotel room after feeling sick during the day. He was known to be treated for epilepsy with Phenhydan®100 mg. A forensic autopsy was performed to exclude an unnatural cause of death.

The following autopsy findings were noted: gingival hyperplasia, ulceration of the laryngeal mucosa, acute lung congestion and enlarged thoracic and abdominal lymph nodes. Tentative autopsy diagnosis was "lymphoma". A detailed histological examination and immunophenotyping were performed on the enlarged lymph nodes. A paracortical expansion by a polymorphous population of immunoblasts (B-cells), plasma cells, histiocytes and cells mimicking Reed-Sternberg cells were seen. Immunophenotyping showed an intact immunoarchitecture and was used to exclude malignant lymphoma. In this case the diagnosis of a phenytoin-associated reactive lymphadenopathy was made. A further discussion of the clinical and histological findings will be presented.

P 08

CONCOMITANT HER2 IMMUNOHISTOCHEMISTRY (IHC) AND IN SITU HYBRIDIZATION (ISH) DETECTION IN GASTROESOPHAGEAL JUNCTION AND GASTRIC ADENOCARCINOMA : BENEFICIAL OR NOT?

Henry P. (1), Vanderveken J. (1), Stevens M. (1), Dubois D. (1), Guiot Y. (1), Jouret-Mourin A. (1) / [1] Cliniques Universitaires Saint-Luc, Brussels,

Introduction:

Background

HER2 analysis in gastroesophageal junction (GEJC) and gastric adenocarcinoma (GC) is required to predict patients' responsiveness to trastuzumab therapy. The current Belgian recommendations for HER2 testing include IHC followed by systematic ISH. Here, we compare the results between IHC and SISH in 221 cases of GEJC and GC.

Methods:

Materials and method

221 cases of GEJC (n=71; 6 poorly cohesive carcinoma (PCC) and 65 intestinal type (IT)) and GC (n=150; 42 PCC and 108 IT), collected over a period of 2 years (01/2013-03/2015), were tested by both IHC (clone 4B5, Ventana) and SISH (brightfield in situ hybridization; Inform Her2) using benchmark Ventana.

Results:

When using concomitant IHC and SISH, HER2 positive rates in GEJC and GC were 29,2% and 17,6% respectively. The concordance of results for HER2 amplification status between IHC and SISH was 95,4% (211/221). Amongst the 10 discordant cases, 9 were false positive results (IHC: 3+ SISH: -), corresponding to 6/25 cases of GC and 3/22 cases of GEJC. Only one false negative result (IHC: 0 SISH: +) was observed in a GEJC case.

Equivocal cases following the European Guidelines (IHC: 2+) were SISH+ in 7/25 of GEJC and 6/47 of GC.

Conclusions:

Some discrepancies were observed when comparing IHC and SISH for HER2 testing.

False positive results by IHC may be caused by misinterpretation of staining or sampling errors, etc. Systematic execution of ISH, as mentioned in the Belgian guidelines, tends to improve the quality of the results.

P 09

MOLECULAR MARKERS FOR INVASIVE UTERINE CERVICAL CANCER: SYSTEMATIC REVIEW

Eerdekens A. (1), Simoens C. (1), Schoolmeesters O. (1), Bogers J. (1) / [1] Laboratory for cell biology and histology, University of Antwerp, Antwerpen

Introduction:

A large number of studies have investigated the occurrence of different tumor-associated antigens on invasive squamous uterine cervical cancer.

Aim:

This systematic review is a preliminary study to distinguish peroperatively invasive uterine cervix tumors from normal cervical squamous epithelium with the aid of tumor-associated antigens. The aim is to produce an evidence based list of tumor-associated antigens specific for invasive squamous uterine cervix tumors and displaying their diagnostic accuracy (sensitivity and specificity).

Methods:

A literature search was performed in Medline (via PubMed), Cochrane Register of Diagnostic Test Accuracy Studies and Web of Knowledge. Only studies that met the predetermined inclusion criteria were included in the analysis. Study quality was assessed using QUADAS (Quality Assessment of Diagnostic Accuracy Studies).

Results:

The literature search resulted in 1012 articles. After selection based on title and abstract 637 articles could be excluded, 323 articles could be excluded after analyzing inclusion criteria. Four articles were included based on expert knowledge and 13 articles remained inaccessible. Data extraction and assessment of study quality was performed for the retained 43 articles. The tumor-associated antigens, described in these 43 articles, were classified according to the six biological properties that tumor cells can obtain during carcinogenesis. For each of these markers the sensitivity and specificity were displayed.

Conclusions:

In this systematic review p16INK4a, survivin, PCNA, Laminin 5 and VEGF were designated as tumor-associated antigens with the best diagnostic accuracy to diagnose invasive squamous uterine cervical tumors and distinguish them from normal cervical epithelium.

P 10

BUILDING A DIGITAL PATHOLOGY ECOSYSTEM FOR EDUCATION AND RESEARCH.

Yves Sucaet, Silke Smeets, Stijn Piessens, Sabrina D'Haese, Chris Groven, Wim Waelput, Peter In't Veld / Department of Pathology, Faculty of Medicine, Vrije Universiteit Brussel

Abstract:

Successful roll-out of digital histopathology requires more than a whole slide scanner. At Brussels Free University (VUB), we currently have two main use cases for whole slide imaging: education and biobanking. Both are presented to various end-users through customized user interfaces. With the help of the Pathomation software platform for digital microscopy, these integrate various datastores and image repositories, where possible. Custom coding is used to interact with various vendor-software and server applications, where needed, and always with the goal of minimal data duplication in mind. The end-result is an interconnected network o heterogeneous information silos, and a thriving environment for multi-disciplinary and integrated (both brightfield and fluorescent) virtual microscopy.
A subset of applications are available to the public via http://www.diabetesbiobank.org.

P 11

AUTOMATED ANALYSIS OF PROLIFERATION AND APOPTOTIC MARKERS IN PAPILLARY THYROID CARCINOMA

Lamba Saini M. (1), Bouzin C. (1), Weynand B. (2), Marbaix E. (1) / [1] Cliniques Universitaires Saint-Luc, Brussels, [2] CHU UCL Mont Godinne, Yvoir

Introduction:

Proliferation and apoptosis are opposing processes by which the cell numbers are kept in a delicate balance, essential for tissue homeostasis, whereas uncontrolled growth of cells is a hallmark of cancer. Papillary thyroid cancer (PTC) is the most common type of thyroid cancer, accounting for 85-90% of all thyroid malignancies. Some PTC seem to follow an indolent course whereas other ones are metastatic and hence, more rapidly progressing tumours. We wanted to investigate whether proliferation and apoptotic markers could help to predict the biological behaviour of different types of PTC.

Aim:

Our aim was to evaluate respective contribution of proliferation and apoptosis in the tumorigenesis of papillary thyroid carcinoma (PTC) by automated analysis. This is the first study of its type which provides an automated assessment of the proliferative capacity and apoptotic potential of cells in PTC, covering the entire spectrum of lesions from 'well differentiated tumour of uncertain malignant potential' (WDT-UMP) to papillary microcarcinoma (PMC), follicular variant of PTC (FVPTC) and finally, metastatic PTC.

Materials and methods:

We investigated the immunolabelling of Ki67, phosphorylated histone H3 (pHH3), cyclin D1, active caspase-3, bcl-2, and p53 in thirteen cases each of metastatic PTC, FVPTC, PMC and WDT-UMP. Slides were scanned followed by digitalization of slides at a 20x magnification by SCN400 slide scanner (Leica, Wetzlar, Germany). The tumour tissue on each slide was delineated manually and scanned slides were then scrutinized using Tissue IA (Leica Biosystems, Dublin, Ireland). Colour deconvolution was applied using haematoxylin and DAB matrices of the software. Nuclear algorithms were applied for Ki67, cyclin D1, p53 and pHH3 immunostaining, keeping the parameters constant for all slides. Cytoplasmic algorithms were applied for bcl 2 and caspase-3 immunolabelling, and the analysis was performed at 20 x magnification.

Results:

Out of the 13 FVPTC cases, seven were encapsulated and six were unencapsulated FVPTC. Ki67 was immunolabelled in more cells of metastatic PTC than of all other types and pHH3 was immunolabelled in more cells of metastatic PTC than of PMC. There was no significant difference between the proportion of cells immunolabelled for Ki67 and pHH3 in the unencapsulated FVPTC and the metastatic PTC. No difference was found for cyclin D1 immunolabelling between the PTC variants. Surprisingly, metastatic PTC and unencapsulated FVPTC also demonstrated more p53 and cleaved caspase-3 immunolabelled cells than other types. In contrast, increased expression of bcl-2 protein was seen in normal thyroid areas, encapsulated FVPTC and PMC as compared to WDT-UMP and metastatic PTC. Metastatic PTC shows higher proliferation than other types of PTC but unexpectedly also higher apoptotic levels. Similar results were also seen with unencapsulated FVPTC, thus demonstrating the fact that unencapsulated FVPTC indeed has a potential for adverse outcome.

Conclusions:

The progress of malignancy can be well tracked by assessing the proliferative/apoptotic number of cells in PTC. The expression of proliferative proteins, Ki67, pHH3 and cyclin D1 in particular, in PTC may indicate an aggressive behaviour by the tumour and loss of apoptosis inhibition by bcl-2 protein can further amplify the role of these proteins in tumour progression. Bcl-2 could prove an interesting marker of PTC precursor lesions. Automated/digital image quantification approach helps in refining the diagnostic accuracy.

P 12

EVOLUTION OF THE IMPLEMENTATION OF A QUALITY MANAGEMENT SYSTEM IN THE BELGIAN LABORATORIES FOR ANATOMIC PATHOLOGY

Verbeke H. (1), Ghislain V. (1), Coucke W. (1), Van Campenhout C. (1), Van de Walle P. (1) / [1] Quality of Medical Laboratories, Scientific Institute of Public Health, Brussels

Introduction:

The Royal Decree (RD) of the 5th December 2011, concerning the licenses of anatomic pathology laboratories came into force the 1th March 2013. The purpose of this RD is to monitor and guarantee the quality of the Belgian laboratories for anatomic pathology. Since the 1th March 2014 all Belgian anatomic pathology laboratories are licensed. Within the framework of this licensing the laboratories are obliged to elaborate a quality management system within five years. However, the requirements concerning topics like access/safety and hygiene, maintenance and calibration of equipment, management of quality documents and method validation, transmission and confidentiality of patient reports, content of patient reports and management and validation of computerized systems as stated in the articles 22, 24, 26, 27, 28 and 29 of the RD, respectively, should be fulfilled within a time period of three months counting from the entry into force of the license. Additionally, participation in the national external quality assessment program organized by the Belgian Scientific Institute of Public Health (IPH) is also required by law.

Aim:

In order to follow up the implementation of the quality management system and the implementation of the articles 22, 24, 26, 27, 28 and 29 of the RD in the Belgian anatomic pathology laboratories, a documentary audit by the department Quality of Medical Laboratories at the IPH has been performed.

Methods:

In 2014, in collaboration with the commission of anatomic pathology, all licensed laboratories were asked to fill out a table in which the percentages of implementation of each article (22/24/26/27/28/29) had to be indicated. In addition, the laboratories were requested to indicate for each item and article the corresponding Standard Operating Procedures (SOPs) and validity date.

In the course of 2015, during a second evaluation step, the laboratories were asked to actualize the table presented in the survey of 2014. In addition, some specific quality documents were requested as well, in particular the patient reports and the SOPs concerning the management of the quality documents and on equipment management.

The tables, patient reports and SOPs were evaluated substantively for the presence of predefined items after which the percentage implementation for each article (only for the evaluation of the tables) and an overall score were calculated.

Results:

From the installation of the RD till 2015, the number of licensed laboratories diminished from 102 in 2013 to 85 in 2015, mostly due to cessation of laboratories of connexists (specialist physicians who perform acts of anatomic pathology exclusively for their own patients).

In 2014, 96 laboratories were included in the evaluation survey and 59 participants (61%) obtained an overall implementation score (all articles includes) of more than 70%. During the second evaluation survey organized in 2015, among the 85 included laboratories, 77 (80%) received an overall implementation score of more than 70%.

In 2014 we counted 16 laboratories (16.6%) with an overall score of less than 25% as compared with 2015, in which no single laboratory scored less than 25%.

More detailed analysis of the obtained information revealed that the procedures on validation of methods and on management and validation of computerized systems seemed to be an issue. In 2014, only 56% of all laboratories had completely implemented the SOP on validation of methods, in contrast with 73% of the laboratories in 2015. In particular the implementation of article 29 (management and validation of computerized systems) seems to remain the biggest obstacle as only 30% of the laboratories had completely implemented this article in 2014 and still one third of all the laboratories are lacking this procedures in 2015.

Conclusions:

The collaboration with the laboratories has contributed to the awareness of the laboratories to work in a quality environment. Close monitoring, adjustment and support by the IPH and the commission of anatomic pathology improved and is still improving the implementation of a quality management system as stated in the RD on licensing conditions of the Belgian laboratories for anatomic pathology.

P 13

CASE REPORT

MALIGNANT TRANSFORMATION OF A PRIOR MULTICYSTIC PERITONEAL MESOTHELIOMA WITH A FOLLOW-UP OF 7 YEARS

Hastir D. (1), Buggenhout A. (1), Simon P. (1), Remmelink M. (1), Noël J. (1) / [1] ULB/Erasme Hospital, Brussels,

Content:

Multicystic peritoneal mesothelioma (MCPM) is a rare lesion occurring most frequently in young women and located in the pelvic mesothelium. This lesion is considered by some authors as a non neoplastic reactionnal mesothelial proliferation but others support that it is a borderline lesion with a tendency to reccur after resection, that could be in rare case responsible of lymph node involvement and more rarely that could underwent malignant transformation. In the present study, we report a case of a 43-year-old female with a previous resected MCPM lesion located in the visceral pelvic peritoneum with an actual local recurrence and a transformation into low grade malignant mesothelioma. Our data are discussed according to a literature review of such rare cases previously described.

P 14

CASE REPORT

INTESTINAL GRAFT LOSS FOLLOWING USAGE OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS.

Steelandt T. (1), Ceulemans L. (1), De Hertogh G. (1), Pirenne J. (1) / [1] UZ Leuven Gasthuisberg, Leuven

Content:

In the last 2 decades, intestinal transplantation has emerged as a valuable life-saving treatment option for patients with intestinal failure and severe complications of total parenteral nutrition. Improved graft survival and quality of life allow recipients to return to their normal daily activities. Due to the unique immunological properties of the intestine, recipients have only a narrow window between infection and rejection. Exogenous factors disturbing the delicate immunologic balance can have a devastating effect. We present a case where the intake of a nonsteroidal anti-inflammatory drug for an orthopedic complaint eventually led to loss of the intestinal graft.

Notification:

This case has already been published in Transplantation in March 2015.

P 15

CASE REPORT

COMBINED KIDNEY AND INTESTINAL TRANSPLANTATION AS A TREATMENT FOR SECONDARY HYPEROXALURIA.

Steelandt T. (1), Ceulemans L. (1), De Hertogh G. (1), Claes K. (1), Monbaliu D. (1), Pirenne J. (1) / [1] UZ Leuven Gasthuisberg, Leuven

Content:

Intestinal transplantation is currently restricted to patients with irreversible small bowel failure who suffer from severe complications of total parenteral nutrition. On the other hand, kidney transplantation is the treatment of choice for end-stage renal disease. Certain intestinal diseases, like extensive intestinal resections, chronic inflammatory bowel disease and other malabsorption syndromes can cause enteric hyperoxaluria which in time will lead to end-stage renal disease. In order to prevent recurrence of the hyperoxaluria induced damage in the transplanted kidney, the transplantation unit of University Hospitals Leuven elected to perform a combined kidney-intestinal transplantation. We present the two cases and highlight the underlying pathophysiological process of hyperoxaluria.

Notification:

This case has already been published in the American Journal of Transplantation in July 2013.

P 16

CASE REPORT

THYROID NODULES: RARE EVENTS OF METASTATIC INVASION OF RCC.

Van Renterghem S., Himpe E., Ruige J. (1), Huvenne W. (1), Van Dorpe J. (1), Praet M. (1) / [1] University Hospital Ghent, Ghent

Content:

Two patients are described, referred to our hospital for a nodule in the thyroid region.

- The first patient, a 67-year-old Caucasian man, presented with an enlarged left lobe with a solid mass with calcification and hypervascularisation. The nodule was confirmed on scintigraphy to be a cold nodule located in the inferior part of the left thyroid lobe. Fine needle aspiration was performed (FNA).

- The second patient, a 71-year-old Caucasian woman, which was in follow-up for a multinodular goiter, developed a suspect nodule in the right thyroid lobe with hypervascularisation on ultrasound (US). Two times FNA was performed.

In both patients, FNA revealed some atypical cells which demonstrated, on immunohistochemisty, expression of CK7, PAX8, CD10 and a negative reaction for TGB and TTF1. The diagnosis of a metastatic location of a renal cell carcinoma was suggested. In both cases, the clinical history did not mention the previous history of RCC. Eventually resection of the nodule was carried out with confirmation of the cytologic diagnosis. In the first patient, the RCC developed 19 years previously; the second patient developed the RCC 16 years priorly.

Review of the literature revealed that metastatic neoplasms to the thyroid gland are rare in clinical practice. CCRC is the most common primary tumor that metastasizes to the thyroid. [1] CCRC metastasizes in an unpredictable manner to organs and can show very late recurrence (up to 20 years). [2] When a patient with a history of cancer presents with a thyroid nodule, metastasis should always be excluded.

[1] Fabio Medas, Pietro Giorgio Calò, Maria Letizia Lai, Massimiliano Tuveri, Giuseppe Pisano and Angelo Nicolosi. Renal cell carcinoma metastasis to thyroid tumor: a case report and review of the literature. Medas et al. Journal of Medical Case Reports 2013, 7:265.

[2] Luca Foppiani, Michela Massollo, Patrizia Del Monte, Roberto Bandelloni, Anselmo Arlandini and Arnoldo Piccardo. Late-Onset Metastasis of Renal Cell Carcinoma into a Hot Thyroid Nodule: An Uncommon Finding Not to Be Overlooked. Case reports in Endocrinology 2015, Article ID 268714.

P 17

CASE REPORT

A CHALLENGING DIAGNOSIS FOR THE PATHOLOGIST: NUT MIDLINE CARCINOMA

Fontanges Q. (1), Heimann P. (1), Salmon I. (1)/ [1] Hôpital Erasme, Brussels

Content:

A 16-year old female has been referred to the hospital for severe cough and sore throat not responsive to antibiotics. Significant past medical history included myeloid acute leukaemia type 5 at the age of 6. Initial work up revealed a 9cm diameter solid mass centered on the right pulmonary hilum associated with multiple mediastinal and hilar lymphadenopathies. An endobronchial ultrasound (EBUS) has been carried out, leading to the diagnosis of blue cell tumour consisting in a poorly differentiated tumour composed of small cells with scant cytoplasm and round hyperchromatic nuclei forming sheets and nests. Tumour cells were focally positive for pan-keratin (AE1AE3) and negative for other epithelial markers (CK7, CK20, p63). Weak and focal expression of CD56 and NSE was observed, without any other neuroendocrine markers antigenicity (chromogranin, synaptophysin, CD57). There was a diffuse membranous expression of CD99. Mesenchymal and lymphoid markers were negative. Final diagnosis was in favour of Ewing/PNET Sarcoma family of tumours.

Nevertheless the EWSR1 translocation was not present, challenging this diagnosis. Clinicians decided to initiate treatment according to the 2008 Ewing protocol (vinc ristine,ifosfamide,doxorubicine,etoposide and radiotherapy). After eleven months the patient presented with motor aphasia and right hemipleagia. Brain MRI showed multiple supratentorial masses radiologically consistent with metastasis. Surgery was performed and pathological examination revealed a highly necrotic blue cell tumour. Immunohistochemical profile was identical to the thoracic tumour specimen. The cytogenetic analysis highlighted that the tumour exhibited highly altered karyotype in which was nevertheless identified a translocation between the long arm of chromosome 15 and the short arm of chromosome 19. This was confirmed by demonstration of a BRD4-NUT fusion transcript by RT-PCR resulting from the balanced translocation t (15; 19) (q14; p13.1).

This translocation is the hallmark of a recently recognised entity: NUT midline Carcinoma (NMC). In 1992 Kuzume and al. described the three first cases of thymic carcinoma with t(15,19) translocation. Since then enough cases have been collected to allow a proper clinicopathological description of the disease. NMC affects mainly young patients and arises in head, neck and mediastinum along the midline axis. It carries a dismal prognosis with a median overall survival of 6.7 months. NMC is a highly challenging diagnosis for pathologist since its morphological and immunohistochemical profile can mimic a wide range of disease. Misleading diagnosis included: sinonasal undifferentiated carcinoma, poorly differentiated squamous cell carcinoma, carcinosarcoma, thymic carcinoma and Ewing/PNET sarcoma family of tumour.

Since 2009, the resulting BRD4-NUT fusion protein nuclear overexpression is detectable by immunohistochemistry with sufficient sensitivity and specificity so that FISH assessment of t(15,19) translocation needs no longer to be performed.

Conventional chemotherapeutic agents are ineffective in treating NMC but nonetheless its unique genetic profile makes it a candidate for emerging targeted therapies, providing a strong rationale for accurate diagnosis of NMC by testing NUT expression in all midline poorly differentiated carcinoma.

P 18

EVALUATION OF SYNOPTIC PATHOLOGY REPORTING OF DUTCH COLORECTAL CARCINOMA

Sluijter C. (1) , Overbeek L. (2), Bos A. (3) , van Slooten H. (4) , Nagtegaal I. (1) / [1]Radboudumc, Nijmegen, Nederlands, [2] Palga, Houten, Nederlands, [3] Netherlands Cancer Registry, , Nederlands, [4] Symbiant, Amstelveen, Nederlands

Introduction:

Traditional narrative pathology reporting (NR) can cause misinterpretation due to lack of information and structure. Therefore, in 2009, the Dutch Pathology Registry (PALGA) introduced synoptic reporting (SR) in the Netherlands initially for colorectal carcinoma (CRC) resections.

Aim:

This study investigated whether the completeness of CRC pathology reports and the quality of gross examination and pathology reports improved after introduction of SR.

Methods:

Pathology data of all CRC patients from 2007 up to 2013 was gathered using the Dutch Cancer Registry (data from NR and SR), and linked to data from PALGA (SR data). Completeness of a pathology report was defined as the proportion of pathology reports in which an individual parameter was present. Quality of CRC gross examination and PA reports was determined by evaluating the percentage of CRC resection specimens with ≥10 lymph nodes (LN) collected and the percentage of pathology reports describing a negative circumferential margin (CRM) respectively and compared between SR and NR (before and after the introduction of SR).

Results:

Data on 66189 CRC was collected, of which 29.6% were rectal carcinomas. 18139 tumours were reported narratively between 2007 and 2008, 28247 tumours were reported narratively between 2009 and 2013, and 19806 tumours were reported synoptically between 2009 and 2013.

Completeness: Before the introduction of SR, histological type, pT-stage, pN-stage and lymph nodes were already reported in more than 98% of the narrative reports. Histological grade and CRM were missing considerably more often, in 17.2% and 27.53% of the pathology reports respectively. With SR, a decrease in the proportion of pathology reports missing an individual parameter was observed for all parameters, especially for CRM, missing only in 2.0% of SR.

Quality: In the period after introduction of SR (2009 up to 2013), the percentage of CRC resection specimens with ≥10 LN collected was higher in SR (86.1%) than NR (75.7%) ($p<0.001$). Furthermore, for rectal carcinomas, a negative CRM was reported more often in SR (91.47%) than in NR before the introduction of SR (63.85%) and in NR after the introduction of SR (80.72%).

Conclusions:

SR appears to increase the probability that mandatory parameters are included in the pathology report, especially for CRM. Furthermore, it appears that the percentage of CRC resection specimens with ≥10 LNs collected increased at least partly due to SR. Additionally, SR seems to positively influence the reporting of CRM. Further analyses are necessary to study the effect of SR on quality of patient care.

P 19

TECHNICAL EVALUATION OF QVINTIP SELF-COLLECTED SAMPLES ON ABBOTT HIGH-RISK HPV AND AML QPCR HPV TEST.

I. Benoy, D. Vanden Broeck, J. Bogers
Department of Molecular Diagnostics, AML, Sonic Healthcare, Antwerp, Belgium
Department of Histopathology, AML, Sonic Healthcare, Antwerp, Belgium

Introduction:

Since HPV testing is considered a valid method of choice for primary cervical cancer screening, the option for non-physician collected samples became a reachable alternative. Many countries, including Belgium, will exploit self-sampling to increase national screening coverage and aim to include hard-to-reach populations by a mail-based prevention approach. The recently introduced self-sampling device Qvintip (Aprovix AB, Sweden) has gained attention due to its limited size and excellent cell yield, and has hence to be considered as an important candidate self-sampling device for future outreach efforts. Qvintip collected samples are stored and shipped in the absence of any medium, prior to laboratory analysis.

Objective:

To evaluate the robustness of Qvintip self-sampling in combination with the AML qPCR HPV test and the Abbott high-risk HPV test.

Methods:

Samples were collected from 12 random female volunteers. Each woman was requested to collect two consecutive samples and carefully mark the order of collection. Collection was done according to the manufacturers information sheet, and no assistance was provided at the time of collection. Upon arrival at the laboratory, sample pairs were randomized into three different categories, and further incubated for 2-3 days. One sample of the pair was always kept at room temperature while the second sample was incubated either at 37°C (arm 1), at 4°C (arm 2), or at room temperature (arm 3). After incubation, DNA was extracted according to Abbott M2000sp specifications, and analyzed with both HR HPV tests (Abbott and AML qPCR).

Results:

Valid results were obtained from all samples, and using both tests. The Abbott test gave an average Cq=20.30 and the AML qPCR Cq=23.26 for beta-Globin. The overall quantitative difference in the beta-Globin Cq between the first collected and the consecutive sample was ΔCq=0.31 (Abbott) and ΔCq=0.32 (AML). The influence of temperature was limited; incubation at 4°C induced a difference in beta-Globin of ΔCq=0.07 (Abbott) and ΔCq=0.20 (AML) and incubation at 37°C of ΔCq=0.65 (Abbott) and ΔCq=0.77 (AML) between paired samples.

Only one sample out of 12 tested positive for HPV, being a triple infection with HPV 35, HPV 59 and HPV 66. No discrepancies were found between the first and second sample at qualitative level for both Abbott and AML test (for AML also at full genotyping level), and only non-significant variation at quantitative level could be noted.

Discussion:

Our preliminary findings suggest that the combination of Qvintip-based self-collected samples and Abbott HR HPV test or AML qPCR test renders a robust configuration. The data also suggest that collection of two consecutive samples is possible without significant loss of DNA content. Furthermore, our data suggest that the temperature at which the self-sampling device is kept, did not induce a significant difference.

NOTES

NOTES

SMART AUTOMATION
powered by sakura

AutoTEC® *a*120
Automated Embedder

- Locked orientation throughout the process
- Prediictable workflow and turnaround time
- Facilitating track & trace of specimen blocks
- Enhanced ergonomics

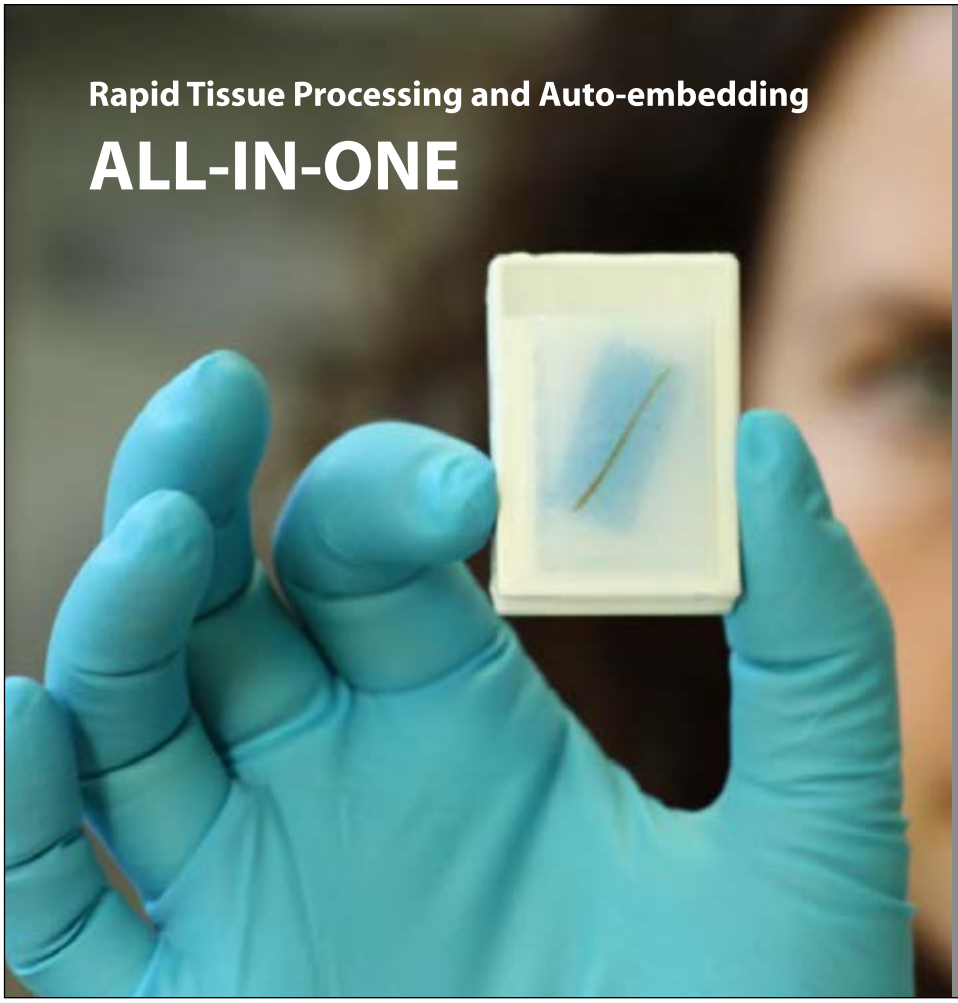
Making the highest quality blocks for you

Building on the success and key principles of SMART Automation, Sakura Finetek launches the second generation fully automated embedder: The Tissue-Tek® AutoTEC® *a*120.
Fully automation of the embedding process provides the highest consistency in block quality as it eliminates need for labor intensive manual handling and retains locked orientation throughout the process from grossing to microtomy.
Only the proven AutoTEC technology combined with the Paraform® Sectionable Cassette System, and new *a*120 features for integrated track & trace of specimen blocks, ensure ultimate patient safety as millions of patients worldwide have experienced until today.

Visit booth C4 for more information

SAKURA

Sakura Finetek Belgium BVBA
autotec.sakura.eu
belgium@sakura.eu



MILESTONE + KLINIPATH
synergy™

KP a VWR Company
KLINIPATH
GOOD(S) IN PATHOLOGY
www.klinipath.be

PARTNERS 2015

THE SUPPORT OF OUR PARTNERS MAKES BWP 2015 POSSIBLE.
THANK YOU !

PREMIUM PARTNERS

Roche

MAJOR PARTNERS

SECTRA
Knowledge and passion

sysmex

Thermo
SCIENTIFIC

REGULAR PARTNERS

ABBOTT
ASTRAZENECA DIAGNOSTICS
BIOCARTIS
BIOKE
DAKO
FRESCO CONSULT
HAMAMATSU
HOLOGIC
LEICA
MEDITHEQUE
MENARINI DIAGNOSTICS
MULTIPLICOM
NIKON
PHILIPS
PROMEGA
PROPATH
SAKURA
VISIOPHARM
VWR