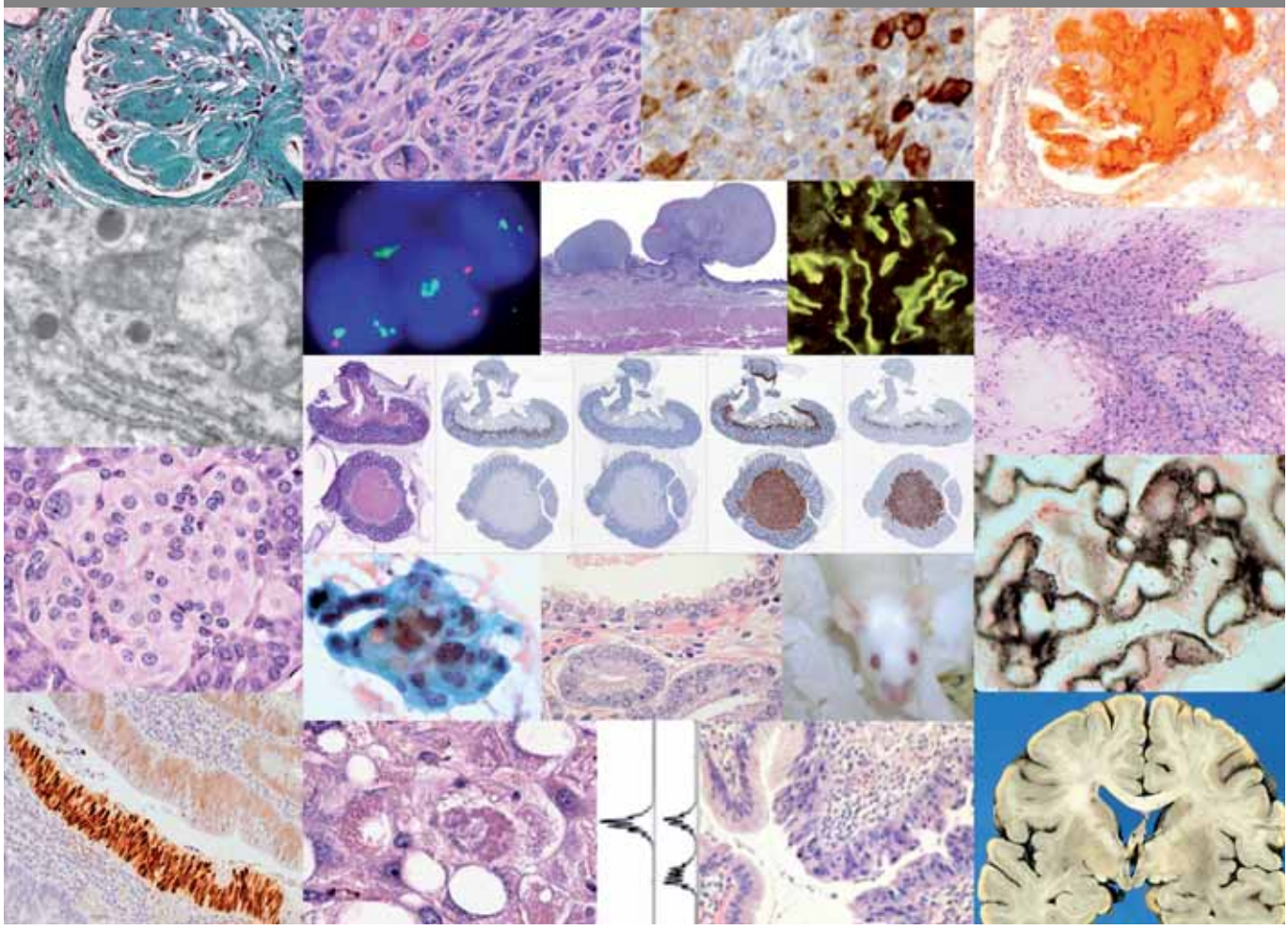
5th BELGIAN WEEK OF PATHOLOGY

OCTOBER 22 - 25, 2014

CONGRESCENTRUM AUGUSTIJNENKLOOSTER - GHENT



www.belgian-society-pathology.eu

WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

SAKURA®

SYMPOSIUM

"LEAN, SMART AUTOMATION AND HYBRID MICROSCOPY"

Speaker: Ms. Olivia Richard

Thursday 23rd of October 2014
13:15 - 14:00

CONTENT:

Sakura has achieved its success and solidified its reputation by providing timely, ingenious solutions to the real challenges laboratories face on a day-to-day basis.
Sakura offer products that anticipate developments in both technology and market need while organizing the laboratories in a LEAN way. We will present you these products and the LEAN principles as well as one of our last innovation: the VisionTek®, which is a surprising hybrid imaging system.

PROGRAM :

LEAN: 10 minutes

SMART Automation: 10 minutes

VisionTek®: 20 minutes

bwpath.be
BELGIAN WEEK OF PATHOLOGY

WELCOME

Dear Colleague,

It is with great pleasure that we invite you to the 5th Belgian Week of Pathology; after the success of the previous meetings, we decided to organize again a 4-day meeting.
This year the venue of the congress has moved to the magnificent Congrescentrum Augustijnenklooster in Ghent, we really hope you will enjoy this new environment!

Pathology is more than ever at the centre of clinical decision making; therefore, the scientific committee has been working on a program to cover the state of the art in scientific knowledge and to address other challenges which will influence our profession in the next years.

We would like to thank our partners from the industry for their renewed support!
Without them, this Week would never be possible…

Take the opportunity to discover the website : **www.belgian-society-pathology.eu** that contains all relevant information on a meeting that brings together world's leading experts, practicing pathologists and basic scientists in one of the nicest cities in Western Europe.

Abstracts were submitted before August 31st. The result of the evaluation was sent to the first author by September 15.
A prize of 2.500€ will be awarded by the Boël Foundation to the Best Oral Presentation addressing research in oncology.
A prize of 500€ will be awarded by the BWP to the Best Poster.

We are looking forward to welcoming you to this 5th Belgian Week of Pathology.

Sincerely yours,

Pieter Demetter - BWP President

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!

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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, 2014.
All abstracts were accepted as:

- Oral presentations and will be presented during the Free paper Session on Friday from 9:00 to 10:00.
- Poster presentations and will be displayed on Thursday and Friday in the Exhibition Area.

The Boël Foundation will award the Best Oral Presentation with a prize of 2.500€
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

Europahotel - Gordunakaaï, 59 - 9000 Ghent
Tel: +32 (0)9 222 60 71 / Fax: +32 (0)9 220 06 09
Monasterium Hotel - Oude houtlei, 56 - 9000 Ghent
Tel: +32 (0)9 269 22 10 / Fax: +32 (0)9 269 22 30

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)
- Belgian Club 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
- Belgian Society of Microscopy

President :	Demetter P.
Past President :	Bogers J.P.
Treasurer :	Hoorens A.
Local Organiser :	Cuvelier C.
Executive Secretary :	D'Haene N.

Councillors :

Dome F.
Jouret-Mourin A.
Salmon I.
Weynand B.
Willocx F.

Thursday
23 October

Symposium
5.30 pm - 6.30 pm

Invitation

"The New Era of Digital Pathology"

TRIBVN
au coeur de l'imagerie numérique

HAMAMATSU
PHOTON IS OUR BUSINESS

Hôpitaux universitaires Paris-Sud
Antoine-Béclère Bicêtre Paul-Brousse
ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

janssen
PHARMACEUTICAL COMPANIES OF Johnson&Johnson

5.30 pm – 5.50 pm
News & Trends in Digital Pathology for 2014

TRIBVN and HAMAMATSU will present the latest trends in digital pathology and reveal the new versions of their products.

Jean-François POMEROL
Medical Imaging Director - TRIBVN
& Quynh-Nhu TRINH-XUAN
Sales Engineer - HAMAMATSU

5.50 pm – 6.10 pm
Feedback on Paris Telepathology Network: frozen section and expertise

Catherine Guettier, who is in charge of the Paris telepathology network, will present main challenges and opportunities around such project.

Catherine GUETTIER
Head of Pathology – Bicêtre University Hospital

6.10 pm – 6.30 pm
epathology empowering the Pathologist

Erio Barale-Thomas will discuss the benefits of e-pathology for the pathologists, using his experience at Janssen.

Erio BARALE-THOMAS
Principal scientist, Pathology, Preclinical Development & Safety – Janssen Research & Development

FOREIGN FACULTY

Arends M.	Cambridge, U.K.
Benbow E.	Manchester, U.K.
Brenn T.	Edinburgh, U.K.
Calonje J.E.	London, U.K.
Carneiro F.	Porto, Portugal
de Leval L.	Lausanne, Switzerland
Di Bonito L.	Trieste, Italy
Egger C.	Lausanne, Switzerland
Fléjou J.-F.	Paris, France
Govindan R.	St Louis, USA
Kerr K.	Aberdeen, U.K.
Kirchner T.	Munich, Germany
Klöppel G.	Munich, Germany
Lauwers G.	Massachussetts, USA
Meijer J.	Arnhem, The Nederlands
Ramsey A.	London, U.K.
Shepherd N.	Gloucestershire, U.K.+
Sobrinho-Simoes M.	Porto, Portugal
Vyberg M.	Aalberg, Danemark
Van de Vijver K.	Maastricht, The Netherlands
Williams G.	Cardiff, U.K.

BELGIAN FACULTY

André J.	Brussels
Bogers John Paul	Antwerp
Bonbled F.	Brussels
Bourgain C.	Bonheiden
Camboni A.	Brussels
Carprieaux M.	Brussels
Colpaert C.	Antwerp
Constante G.	Brussels
Cornelis A.	Tienen
Creytens D.	Ghent
Cuvelier C.	Ghent
D'Haene N.	Brussels
de Saint Aubin N.	Brussels
De Schepper S.	Ghent
Demetter Pieter	Brussels
Dome F.	Liège
Geboes K.	Ghent
Hoorens A.	Brussels
Jouret-Mourin Anne	Brussels
Kokx M.	Antwerp
Le Mercier M.	Brussels
Moerman P.	Leuven
Moles Lopez X.	Brussels
Noël J.-C.	Brussels
Pauwels P.	Antwerp
Praet M.	Ghent
Remmelink M.	Brussels
Salmon I.	Brussels
Sciot R.	
Segers V.	Brussels
Slabbaert	Brussels
Sokolow Y.	Brussels
Somja J.	Liège
Théate I.	Gosselies
Theunis A.	Brussels
Van Eycken	Brussels
Weynand B.	Yvoir
Willocx F.	Brussels

Dermatologie:
Sofie De Schepper
Anne Theunis

PROGRAM OVERVIEW

		KLOOSTERGANGEN Ground floor	AUGUSTIN ROOM Ground - 200 pax	HIPPO/CARTHAGUE ROOM Ground - 120 pax
WEDNESDAY October 22	14:00 - 14:05		Opening : P. Demetter	
	14:05 - 15:30		Postgraduate Course : Autopsy pathology	
	15:30 - 16:00	Coffee Break Ehibition Area		
	16:00 - 17-30		Postgraduate Course : Autopsy pathology	
THURSDAY October 23	9 :00 - 10 :30	Exhibition Area Posters	Dermatopathology: Cutaneous soft tissue tumors	Ethics and Economy: Telepathology
	10:30 - 11:00	Coffee Break Exhibition Area/Posters		10:30 – 11:00 General Assembly Belgian Society Pathology
	11:00 - 12:00	Exhibition Area Posters	Dermatopathology: Cutaneous soft tissue tumors	Ethics and Economy: Telepathology
	12:00 - 12 :45	Exhibition Area Posters	Keynote Lecture: M. Sobrinho-Simoes Porto, Portugal	
	12:45 - 14:00	LUNCH Exhibition Area/Posters	13 :15-14 :00 Satellite Symposium SAKURA	
	14 :00 - 15 :30	Exhibition Area Posters	Pulmonary pathology	Thyroid histopathology and cytology
	15:30 - 16:00	Coffee Break Exhibition Area		
	16:00 - 17:30	Exhibition Area Posters	Pulmonary pathology	Thyroid histopathology and cytology
	17 :30-18 :30	Exhibition Area Posters	Satellite Symposium HAMAMATSU/TRIBVN The new era of digital pathology	
	18 :30-20 :00	Cheese & Wine		

		KLOOSTERGANGEN Ground floor	AUGUSTIN ROOM Ground - 200 pax	HIPPO/CARTHAGUE ROOM Ground - 120 pax
FRIDAY October 24	8:55 - 10:00	Exhibition Area Posters	Inflammatory pathology of the digestive tract	9:00 – 10:00 Free paper Session
	10:00 - 10:30	Coffee Break Exhibition Area/Posters		
	10:30 - 12:00	Exhibition Area Posters	Inflammatory pathology of the digestive tract	
	12:00 - 13:00	Exhibition Area Posters	Keynote Lecture F. Carneiro/ Porto, Portugal	
	13:00 - 14:00	LUNCH Exhibition Area/Posters	Satellite Symposium ROCHE	
	14:00 - 15:30	Exhibition Area Posters	Oncology of the digestive tract	Pathology of the endometrium
	15:30 - 16:00	Coffee Break Exhibition Area/Posters		
	16:00 - 17 :30	Exhibition Area Posters	Oncology of the digestive tract	Pathology of the endometrium
	17:30 - 17:45		BWP 2014 Awards : Boël Prize / Best Poster	
SATURDAY October 25	9:00 - 10 :30	Exhibition Area	Haematopathology: Extranodal and splenic lymphomas	Program for Cytotechnologists
	10:00 - 10:30	Coffee Break Exhibition Area		
	10:30 - 12:00	Exhibition Area	Haematopathology: Extranodal and splenic lymphomas	Program for Cytotechnologists
	12:00 - 13:00	Exhibition Area	The National Cancer Registry and the pathologists: a win-win collaboration?	
	13:00 - 14:00	LUNCH Exhibition Area		
	14:00 - 16:00	Exhibition Area	Pigmented skin lesions	
	16:00 - 16:05	Exhibition Area	Closing: P. Demetter	

Roche Satellite & Lunch Symposium

During the Belgian Week of Pathology

- New Belgian guidelines for HER2 testing: Dr. C. Colpaert
- Overview of available anti-HER2 therapies: Prof. dr. H. Denys

Friday 24TH October 2014 from 13h00 till 13h55

Congrescentrum Augustijnenklooster - Gent

This is what HER2 testing is all about and what we will discuss during the Roche satellite symposium at the Belgian week of Pathology.

Dr. C. Colpaert will guide you through the recently published Belgian guidelines on HER2 testing. Implementing these guidelines will increase the HER2 testing quality which directly affects many (patient)lives. The guidelines were developed by the Belgian HER2 taskforce and are based on the ASCO/CAP guidelines published last year.

Prof. Dr. H. Denys, as an experienced oncologist, will give a clear overview on the available anti-HER2 therapies used in the clinic.
She will address the clinical value of the different therapies and explain why pathologists play a vital role in the treatment decision.

Accurate HER2 testing enables an adapted and potentially life saving treatment decision for the patient.

Program

Chair: Dr. K. Lambein (UZ Gent)

13h00-13h30: Dr. C. Colpaert (Sint Augustinus, Antwerp)

13h00-13h25	New Belgian guidelines for HER2 testing
13h25-13h30	Q&A

13h30-13h55: Prof. dr. H. Denys (UZ Gent)

13h30-13h50	Importance of accurate HER2 testing in the light of the newly available anti-HER2 therapies: an oncologists' perspective
13h50-13h55	Q&A

R. E.: Dr. Chr. Lenaerts - Br 1137 - 11/08/2014

Congrescentrum Augustijnenklooster - Academiestraat 1 - 9000 Gent

WEDNESDAY 22 AFTERNOON

Room AUGUSTIN ROOM

14:00-14:05 : **Welcome message** *P. Demetter (Brussels)*

Room AUGUSTIN ROOM

14:05-17:30 : **Postgraduate course: Autopsy pathology.**
Chairpersons : *F. Bonbled (Brussels), N. D'Haene (Brussels)*

14:05-14:45	**The non-forensic autopsy: is it still valuable?** *(E. Benbow, Manchester, U.K.)*
14:45-15:30	**Partial autopsy, forensic autopsy and cardiac dissection.** *(F. Bonbled, Brussels)*
15:30-16:00	Coffee break
16:00-16:45	**Conventional autopsy and post-mortem imaging : advantages, limitations and complementarity.** *(C. Egger, Lausanne, Switzerland)*
16:45-17:30	**Specificities of the macroscopic fetal autopsy.** *(V. Segers, Brussels; N. D'Haene, Brussels)*

THURSDAY 23 MORNING

Room AUGUSTIN ROOM

09:00-12:00 : **Dermatopathology: Cutaneous soft tissue tumors.**
Chairpersons : *A. Theunis (Brussels), N. de Saint Aubain (Brussels)*

09:00-10:00 **Update on cutaneous soft tissue tumours.**
(T. Brenn, Edinburgh, U.K.)
10:00-10:30 **Slide seminar.**
(T.Brenn, R. Sciot, D. Creytens, J. André, N. de Saint Aubain, A. Theunis)
10:30-11:00 Coffee break
11:00-12:00 **Slide seminar.**
(T.Brenn, R. Sciot, D. Creytens, J. André, N. de Saint Aubain, A. Theunis)

Room HIPPO/CARTHAGUE ROOM

08:30-12:00 : **Ethics and economy: Telepathology.**
Chairpersons : *J.P. Bogers (Antwerp), X. Moles Lopez (Brussels)*

08:30-09:00 **Are you ready for digital pathology?**
(X. Moles Lopez, Brussels)
09:00-09:30 **Use of digital pathology in quality assessment.**
(M. Vyberg, Aalborg, Denmark)
09:30-10:00 **Digital pathology in clinical pathology: the Belgian experience.**
(A. Cornelis, Tienen)
10:00-10:30 **Virtual microscopy as a tool for pharmacodiagnostic analyses in solid tumours.**
(M. Kockx, Antwerp)
10:30-11:00 Coffee break
11:00-11:30 **Digital pathology in second revision of rare tumours.**
(I. Salmon, Brussels)
11:30-12:00 **New digital horizons for Palga, the dutch database of pathology.**
(J. Meijer, Arnhem, The Netherlands)

Room HIPPO/CARTHAGUE ROOM

10:30-11:00 : **General Assembly Belgian Society of Pathology.**
Chairpersons : *J.P. Bogers (Antwerp), X. Moles Lopez (Brussels)*

Room AUGUSTIN ROOM

12:00-12:45 : **Keynote lecture.**
Chairpersons : *I. Salmon (Brussels)*
How to diagnose malignancy in well differentiated thyroid tumours?
(M. Sobrinho-Simoes, Porto, Portugal)

Room AUGUSTIN ROOM

13:15-14:00 : **Satellite Symposium SAKURA**
"LEAN, SMART Automation and hybrid microscopy""
Olivia Richard
LEAN: 10 min. - SMART Automation: 10 min.- VisionTek®: 20 min.

THURSDAY 23 AFTERNOON

Room HIPPO/CARTHAGUE ROOM

14:00-17:00 : **Thyroid histopathology and cytology.**
Chairpersons : *I. Salmon (Brussels), Y. Sokolow (Brussels)*

14:00-14:30 **Cytology of thyroid punctures.**
(M. Carprieaux, Brussels)
14:30-15:00 **Rare tumours of the thyroid.**
(M. Sobrinho-Simoes, Porto, Portugal)
15:00-15:30 **Second opinion in rare thyroid cancers.**
(G. Costante, Brussels)
15:30-16:00 Coffee break
16:00-16:30 **Application of next generation sequencing to fine needle aspiration.**
(M. Le Mercier, Brussels)
16:30-17:00 **Frozen sections in thyroid pathology.**
(Y. Sokolow, Brussels)

Room HIPPO/CARTHAGUE ROOM

14:00-17:30 : **Pulmonary pathology.**
Chairpersons : *B. Weynand (Yvoir), M. Remmelink (Brussels)*

14:00-15:00 **WHO classification of Lung Cancer: New look.**
(K. Kerr, Aberdeen, U.K.)
15:00-15:30 **ALK immunohistochemistry - Belgian guidelines.**
(P. Pauwels, Antwerp)
15:30-16:00 Coffee break
16:00-17:00 **Clinical Implications of Cancer Genome Sequencing.**
(R. Govindan, St. Louis, USA)
17:00-17:30 **Interstitial pneumopathies: new Belgian working group and slide seminar.**
(M. Remmelink, Brussels and B. Weynand, Yvoir)

Room HIPPO/CARTHAGUE ROOM

10:30-11:00 : **Satellite Symposium HAMAMATSU and TRIBVN :**
"The new era of digital pathology."
17:30-17:50 **Introduction, News and Trends in Digital Pathology.**
Quynh-Nhu TRINH-XUAN, HAMAMATSU & Jean-François POMEROL, TRIBVN
17:50-18:10 **Feedback on Paris Telepathology Network : frozen section and expertise.**
Catherine GUETTIER, Head of Pathology, University Hospital Bicêtre, Paris
18:10-18:30 **ePathology empowering the pathologist.**
Erio BARALE-THOMAS, Principal scientist, pathology, Preclinical development & Safety, Janssen research & development, A division of Janssen Pharmaceutica NV

18:30-20:00 : **Reception: Cheese and Wine**

FRIDAY 24 MORNING

Room AUGUSTIN ROOM

08:55-11:30 : **Inflammatory pathology of the digestive tract.**
Chairpersons : *A. Jouret-Mourin (Brussels), P. Demetter (Brussels)*

08:55-09:00 **Introduction.**
(P. Demetter / Brussels)
09:00-09:30 **Update on inflammatory diseases of the oesophagus.**
(J. - F. Fléjou / Paris, France)
09:30-10:00 **Update on inflammatory diseases of the stomach.**
(G. Lauwers, Massachusetts, USA)
10:00-10:30 Coffee break
10:30-11:00 **Update on inflammatory diseases of the small intestine.**
(G. Williams, Cardiff, U.K.)
11:00-11:30 **Update on inflammatory diseases of the colon.**
(N. Shepherd, Gloucestershire, U.K.)

Room HIPPO/CARTHAGUE ROOM

09:00-10:00 : **Free paper session.**
Chairpersons : *N. D'Haene (Brussels), C. Colpaert (Antwerp)*

9h00 **O1** AUTOPSY CASE REPORT: INTRA-EPITHELIAL LYMPHOCYTES, VILLOUS ATROPHY, FOAMY MACROPHAGES AND BEYOND...
E. Bodson (1), S. Verschuere (2), G. Vanneste (3), L. Libbrecht (2), M. Praet (2), K. Geboes (2) / [1] AZ Sint Jan, Brugge; [2] UZ Gent; [3] AZ Groeninge, Kortrijk

9h10 **O2** IGG4 EXPRESSION IN LABIAL SALIVARY GLANDS IN PATIENTS WITH SICCA SYNDROME.
A. Goubella (1), M.S. Soyfoo (1), M. Remmelink (1), J.L. Nortier (1), A. Pozdzik (1) / [1] Hôpital Erasme, Bruxelles

9h20 **O3** IMPACT OF NEOADJUVANT THERAPY ON CANCER-ASSOCIATED FIBROBLASTS IN RECTAL CANCER.
Laurine Verset (1), Joke Tommelein (2), Xavier Moles Lopez (3), Christine Decaestecker (3), Tom Boterberg (2), Isabelle Salmon (1,3), Marc Mareel (2), Marc Bracke (2), Olivier De Wever (2), Pieter Demetter (1*) / [1] Erasme Hospital,(ULB), Brussels, Belgium; [2] Universiteit Gent, Belgium (UG), [3] DIAPATH-CMMI, Gosselies, Belgium*

FRIDAY 24 MORNING

9h30 **O4** EXPRESSION OF MAP KINASES IN PAPILLARY THYROID CARCINOMA.
M. Lamba Saini (1), B. Weynand (2), M. Mourad (1), M. Hamoir (1), E. Marbaix (1) / [1] Cliniques Universitaires Saint-Luc, Bruxelles; [2] CHU UCL Mont Godinne, Yvoir

9h40 **O5** PARACRINE REGULATION OF STROMAL PROTEIN EXPRESSION IN BREAST AND COLORECTAL CANCER.
M. Van Bockstal (1), K. Lambein (1), M. Van Gele (1), G. Braems (1), V. Cocquyt (1), H. Denys (1), M. Bracke (1), L. Libbrecht (1), O. De Wever (1) / [1] UZ Gent, Gent

9h50 **O6** CASE REPORT: RARE SOFT PLAQUE DISEASE CAN MYSTIFY STAGING OF COLON CARCINOMA.
E. Bodson (1) / [1] AZ Sint Jan, Brugge

Room AUGUSTIN ROOM

12:00-13:00 : **Keynote lecture.**
Chairpersons : *A. Jouret-Mourin (Brussels), K. Geboes (Ghent)*
Update on gastric cancer.
(F. Carneiro, Porto, Portugal)

Room AUGUSTIN ROOM

13:00-14:00 : **Satellite Symposium ROCHE.**

13:00-13:30 : *Dr. C. Colpaert (Sint Augustinus, Antwerp)*
13:00-13:25 : New Belgian guidelines for HER2 testing
13:25-13:30 : Q&A
13:30-13:55 : *Prof. dr. H. Denys (UZ Gent)*
13:30-13:50 : Importance of accurate HER2 testing in the light of the newly available anti-HER2 therapies: an oncologists' perspective
13:50-13:55 : Q&A

FRIDAY 24 AFTERNOON

Room AUGUSTIN ROOM

14:00-17:30 : **Oncology of the digestive tract.**
Chairpersons : *C. Cuvelier (Ghent), A. Hoorens (Brussels)*

14:00-14:45 **Carcinogenesis and molecular subtyping of sporadic colorectal carcinoma.**
(T. Kirchner, Munchen, Germany)
14:45-15:30 **The molecular pathology of hereditary colorectal cancer.**
(M. Arends, Cambridge, U.K.)
15:30-16:00 Coffee break
16:00-16:45 **The genesis of pancreatic tumours.**
(G. Klöppel, Munchen, Germany)
16:45-17:30 **Dysplasia in the oesophagus.**
(J.-F. Fléjou, Paris, France)

Room HIPPO/CARTHAGUE ROOM

14:00-17:30 : **Pathology of the endometrium.**
Chairpersons : *P. Moerman (Leuven), C. Colpaert (Antwerp)*

14:00-14:45 **The non-neoplastic endometrial biopsy.**
(P. Moerman, Leuven)
14:45-15:30 **Precursor lesions of endometrial carcinoma.**
(J.-C. Noël, Brussels)
15:30-16:00 Coffee break
16:00-16:45 **Histological typing of endometrial carcinoma.**
(C. Colpaert, Antwerp)
16:45-17:30 **Grading, staging and molecular aspects of endometrial carcinoma.**
(K. Van de Vijver, Amsterdam, The Netherlands)

SATURDAY 25

Room HIPPO/CARTHAGUE ROOM

09:00-12:00 : **Program for cytotechnologists.**
Chairpersons : *F. Willocx (Brussels), F. Dome (Liège)*

09:00-09:30 **New approach and automation in urinary cytology.**
(C. Bourgain, Bonheiden)
09:30-10:00 **Squamous lesions and their look-alikes.**
(L. Di Bonito, Trieste, Italy)
10:00-10:30 Coffee break
10:30-11:00 **Glandular lesions and their look-alikes.**
(L. Di Bonito, Trieste, Italy)
11:00-12:00 **Slide seminar.**

Room AUGUSTIN ROOM

09:00-12:00 : **Haematopathology: Extranodal and splenic lymphomas.**
Chairpersons : Somja (Liège), A. Camboni (Brussels)

09:00-09:30 **Splenic lymphomas.**
(L. de Leval, Lausanne, Switzerland)
09:30-10:00 **MALT lymphoma in gastrointestinal tract.**
(A. Camboni, Brussels)
10:00-10:30 Coffee break
10:30-11:00 **Primary lymphoma of the female genital tract.**
(A. Ramsay, London, U.K.)
11:00-12:00 **Slide seminar.**

Room AUGUSTIN ROOM

12:00-13:00 : **The National Cancer Registry and the pathologists: a win-win collaboration?**
Chairpersons : *C. Cuvelier (Ghent), P. Demetter (Brussels)*

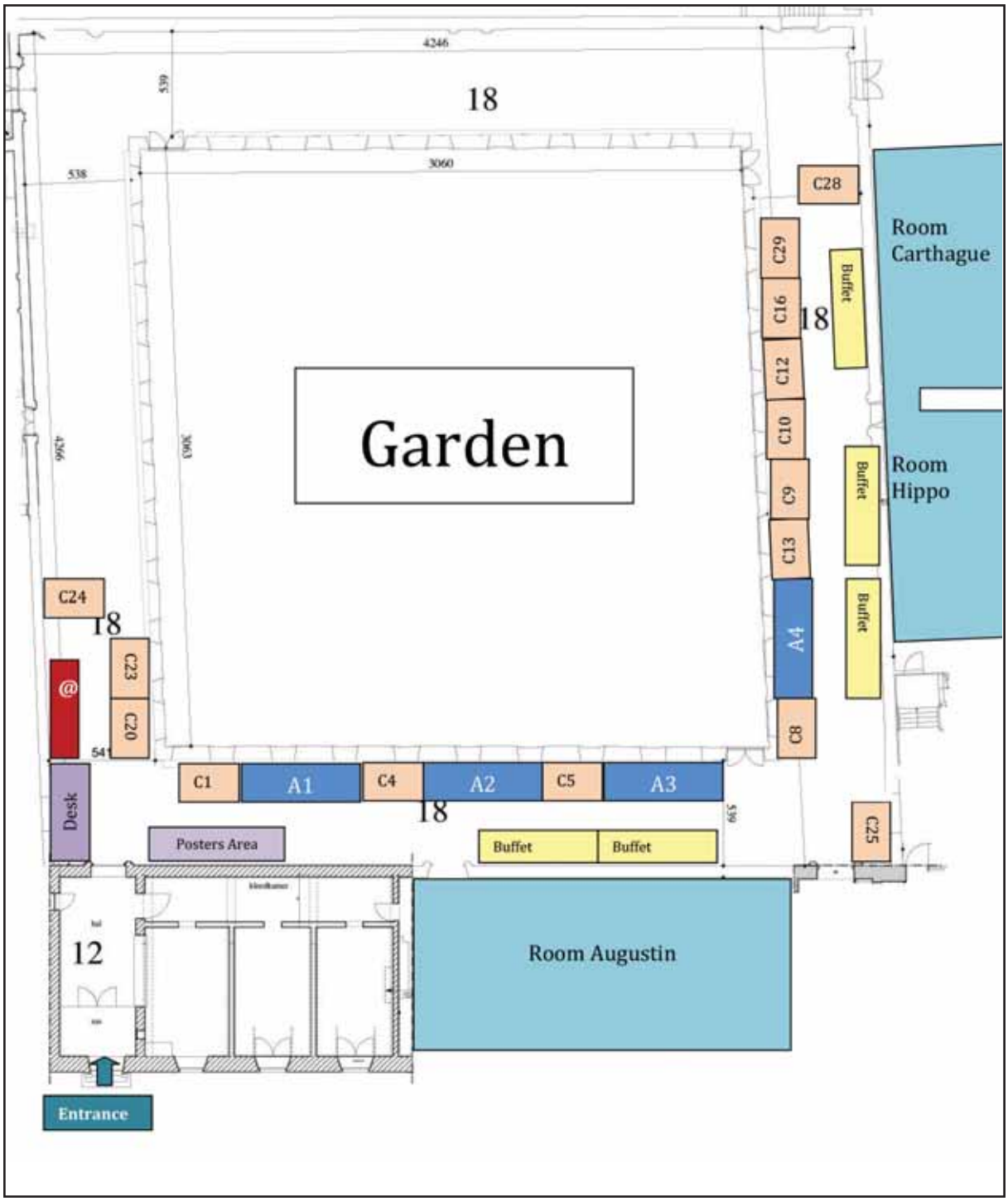
12:00-12:30 **Minimal data set to obtain optimal registration data from pathologists.**
(M. Slabbaert, Brussels)
12:30-13:00 **How the registry data help in the research on the Belgian population.**
(E. Van Eycken, Brussels)

Room AUGUSTIN ROOM

14:00-16:00 : **Pigmented skin lesions.**
Chairpersons : *D. Creytens (Ghent), S. De Schepper (Ghent)*

14:00-15:00 **Pigmented skin lesions: state of the art.**
(J.E. Calonje, London, U.K.)
15:00-16:00 **Slide seminar.**

EXHIBITION FLOOR



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	C 16	SYSMEX
	C 20	HAMAMATSU
	C 23	BIO BANKS: CMI / BWB
	C 25	ABBOTT - 1day Thursday
	C 29	CEPHEID

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INVITED LECTURES

L 01
The non-forensic autopsy: is it still valuable?

Dr Emyr W Benbow
Senior Lecturer in Pathology, University of Manchester, UK
Honorary Consultant Pathologist, University of Manchester, UK

The role of the autopsy has changed over decades, and some would claim that it now has little relevance beyond the investigation of alleged crime, and even in that field, imaging methodologies such as CT scanning have made substantial inroads. In England, Wales and Northern Ireland (Scotland has a different legal system), almost all autopsies are carried out at the instruction of the Coroner: the trend for the disappearance of autopsies carried out with the consent of the family started a long time ago,[1] and has continued. Strictly speaking, all autopsies performed for legal reasons (and the Coroner's role is a legal one) are "forensic", but by convention, the term "forensic autopsy" is limited to those with a potential to lead to a criminal investigation.
The Coroner investigates all other unnatural deaths by holding an inquest, usually after an autopsy, and he or she is also responsible for investigating deaths that were sudden and unexpected: both these categories are designated as "non-forensic" in the UK.

There has been much debate about the value of autopsies in the UK, following the discovery of widespread retention of material from the deceased, without the knowledge and consent of the relatives, about 15 years ago. This has led to changes in our laws, though it is not yet clear whether these changes have been of benefit; in a number of ways, they impede the purposes that I think that are most important.

Not only are autopsies significant for these legal reasons, they are important for the information they provide to

- the relatives of the deceased
- the clinicians who cared for the patient in life
- the nation and society

The relatives of the deceased
Cultural and other factors influence the degree of importance placed on knowing the cause of death, and religious ritual may take precedence; the disease causing death may be of no interest to a community that believes that death is the will of a higher being, or that death is simply the consequence of age. However, even remote communities with strict beliefs about rapid disposal of dead bodies can appreciate the value of modified autopsies in identifying infectious disease.[2] In more sophisticated cultures, there is a clear understanding of the value of autopsy, especially in the investigation of inheritable diseases: the CRY Centre for Cardiac Pathology in London was established by charitable funds, and aims to provide a morphological diagnosis following sudden unexpected death within two weeks of the receipt of the subject's heart.[3]

The clinicians who cared for the patient in life

There have been many studies of the rate of discrepancy between clinical diagnosis and autopsy diagnosis, accompanied by pleas to make use of this resource as a rich source of audit data. These have been characterised by a surprisingly slow and irregular reduction in the rate of discrepancy, though it is clear that some commentators have failed to understand that the denominator in these rates has changed significantly.[4] A detailed epidemiological study has shown a slow reduction in the discrepancy rate, though this required careful mathematical extrapolation to extract a trend from the statistical noise.

Some have ascribed the plummeting numbers of non-forensic autopsies to a belief amongst clinicians that there is little left to be learned after death because of the sophistication of investigation in life. This is in sharp contrast to the published research from clinical groups such as intensive care practitioners, who are intimately familiar with modern diagnostic methods: a recent systematic review extrapolates from autopsy data that diagnostic errors lead to 40500 deaths in ICU patients in the USA each year.[6] Non-autopsy studies also indicate high rates of diagnostic error in patients who die: a case note review of 14000 admissions in Australia revealed adverse events in 16.6% of them, of which 4.9% contributed to death.[7]

The nation and society

Good health care requires accurate statistics on the serious disease prevalent in each community, and it is self-evident that this requires comprehensive information on the diseases that cause death. Much of this information is based on systems of death certification, and it is clear that much of it is poor: a World Health Organisation study of cardiovascular diseases found that some countries have such a high rate of "ill-defined" causes of death, such as "cardiac arrest" or "myocardial degeneration" that complex mathematical formulae have to be applied before comparative data can be derived.[8] Cause-of-death statements, even when the diagnoses are correct, are often filled out so badly that statistical coding is impeded: pathologists can provide accurate causes of death, and are much more adept than clinicians at completing cause-of-death statements coherently.[9] Better completion of death certificates has a major role to play in direct monitoring of issues such as the contribution of adverse therapeutic outcomes to death: a study in New York City shows that only one in every fourteen cases where an adverse medical event had contributed to death had been reported as such.[10]

The autopsy remains an important tool in maintaining the quality of health care: the dead can continue to teach the living.

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L 03

Conventional autopsy and postmortem imaging: Advantages, limitations and complementarity.

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Since the earliest years after the discovery of X-rays, radiological images have been successfully used for answering medico-legal and forensic questions. The possibility to evaluate the inside of a body without actually opening it has been appreciated and used in forensic pathology since then.

While the development of postmortem imaging was slower than that of clinical imaging, we are currently witnessing an exponentially increasing use of modern imaging techniques in forensic pathology, and to a lesser extent in clinical pathology. Unfortunately, the introduction of cross-sectional imaging techniques into post-mortem investigations has created controversial discussions among the medico-legal community. Terms like “Virtual Autopsy” and “Necro-Radiology” have led to confusion and controversy concerning the role of radiological techniques in forensic case work.

Regardless, the use of those techniques in post-mortem investigations is increasing, especially the one of Muti-Detector Computed Tomography (MDCT). The proper interpretation of postmortem images requires a close collaboration between pathologists and radiologists: the radiologist is used to interpret clinical images, but the knowledge of forensic pathology is fundamental to recognize postmortem artifacts and establish a correct interpretation, diagnosis and conclusion. This interdisciplinary gap in conjunction with the rapid increase of post-mortem imaging has triggered the development of new subspecialties, such as forensic radiographers, pathologists trained in post-mortem imaging and radiologists familiar with post-mortem images.

Different kinds of post-mortem artifacts are encountered and must be known when reading radiological post-mortem images. The most frequent is the presence of gas. Its detection can be essential in some cases, e.g. the diagnostic of vital air embolism versus putrefaction gas. Studies have been performed in order to be able to differentiate a vitality phenomenon from a decomposition process [1,2].

Although PMCT allows visualizing the skeletal system in excellent quality, soft tissue information is limited. Especially the interpretation of the vascular system and the inner organs is difficult. Lesions can often be suspected e.g. due to the presence of

surrounding blood, but only rarely directly visualized. For these reasons, other imaging techniques such as Postmortem Angiography and Magnetic Resonance Imaging (MRI) are increasingly applied. Indeed, MDCT has only a limited diagnostic value for investigating cases of natural death, as in these cases soft tissues and vessels are the most important structures to be explored, especially as cardiovascular diseases represent the most common cause of death in developed countries.- In order to overcome these limitations, , different techniques for post-mortem CT-Angiography have been developed. The injection of a contrast agent into the vascular system renders the lumen of vessels visible and soft tissue, such as organ parenchyma is enhanced, allowing a better exploration of those structures.

The recently developed technique of Multi-phase Post-Mortem CT-angiography (MPMCTA) is a method of minimally-invasive whole-body CT angiography which aims to increase the sensitivity of the radiological examination [3-4]. By applying the standardized protocol of MPMCTA, the vessels of the head, thorax and abdomen can be depicted in their entirety, allowing a detailed investigation of all vessels, especially the coronary arteries. The application of contrast agent also allows the investigation of inner organs and the identification of parenchyma lesions. Especially the myocardium can be explored much more easily, as the filling of the cardiac cavities with contrast agent allows a better distinction of the anatomy and in some cases even the identification of cardiac infarction.

By applying the technique of MPMCTA, the majority of cases of natural death can even be solved before autopsy. Best examples are deaths due to aortic dissections, coronary artery disease, coronary thrombosis, cerebral or cardiac infarction and cerebral and other internal hemorrhages. The diagnosis of pulmonary artery embolism remains difficult due to the regular presence of post-mortem blood clots in the pulmonary arteries which are mimicking real pulmonary embolism [5]. In order to solve this problem, ongoing studies try to establish rules for the radiological interpretation of MPMCTA cases that should allow the distinction between those artifacts and real pathological findings in the near future.

The use of MPMCTA as a method for quality control in hospitals has recently been studied by Wichmann et al. [6]. This study compared MPMCTA to medical autopsy in 50 hospitalized patients who died unexpectedly or within 48 hours of an event necessitating cardiopulmonary resuscitation. MPMCTA confirmed 93% of all 336 diagnoses identified from ante-mortem medical records, and medical autopsy confirmed 80%. In addition, MPMCTA and medical autopsy identified 16 new major and 238 new minor diagnoses. Seventy-three of the virtual autopsy diagnoses, including 32 cases of coronary artery stenosis, were identified solely by MPMCTA. This led to the conclusion that in cases of unexpected death, the addition of MPMCTA increases the value of native PMCT, making it a feasible alternative for quality control and identification of diagnoses traditionally made by medical autopsy.

MRI delivers excellent soft tissue contrast and is thus very useful for the investigation of deep soft tissues or parenchyma lesions. Common applications are for example cases of natural death, especially sudden cardiac death, all types of soft tissue cancer or cases in which contusion wounds of the soft tissue are important such as traffic accidents.

In summary, this presentation will discuss the role of postmortem imaging in order to distinguish between real state of the art and virtual reality. It shall give an overview over the different techniques of postmortem imaging. It will critically explain their advantages and limitations in post-mortem investigations compared to conventional autopsy.

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INVITED LECTURES

L 04
Specificities of the macroscopic fetal autopsy.

Valérie Segers, *Laboratory of Pathology, Centre Hospitalier Universitaire Brugmann, Brussels, Belgium*
Nicky D'Haene, *Laboratory of Pathology, Erasme Hospital, Brussels, Belgium*

The major objectives of the fetal autopsy are to document growth and development, determine gestational age, detect malformations, evaluate diagnoses and determine the cause of the death. The approach to the fetal autopsy differs from that of older people. Both maternal health and the intrauterine environment must be evaluated which include placental examination.
The first step of this approach is the macroscopic examination, which is the subject of this talk, followed by microscopic examination, integration with clinical data (prenatal diagnosis, familial history, genetic results,…) in order to provide an integrated summary of the findings.

The routine autopsy follows a systematic approach using an established protocol. The use of checklists insures the efficient and complete recording of findings.
Before autopsy, a whole body X-ray is required for gestational assessment, malformations or skeletal dysplasia.
The first step is to take photographs. It is a necessary part of routine examination, providing indisputable evidence of findings (or the absence of findings). In our practice, we take for all autopsies these minimal photographs: the whole body, the face (face and profiles), hands, feets and the external genitalia. Any abnormal finding is also photographed.
The second step is to take weight and measurements, essential to document growth and development. Measurements include crown-heel and crown-rump lengths, head circumference, foot length which correlates especially well with gestational age.
External examination parallels the physical examination performed in clinical settings. The examination is carried out systematically from one end of the body to the other, and from anterior to posterior. The distribution and severity of maceration or oedema are noted. Dysmorphic features were evaluated (implantation of the ears, palpebral length, distance between inner canthi, nose, tongue appearance), as for the presence or absence of orofacial clefting, abdominal wall abnormalities (gastroschisis, omphalocoele), back abnormalities (spina bifida). The external genitalia are noted. The 4 limbs are also evaluated and the digits are counted with an evaluation of syndactyly.
The internal examination begins with the documentation of the presence of the diaphragm and the evaluation of the situs. The presence of a lung hypoplasia is evaluated. The position, calibre, and rotation of each component of the gastrointestinal tract are ascertained. The position and the size of the liver and the spleen are noted.

The size of the urinary bladder is evaluated. The internal genitalia are inspected. After all pertinent information is gained from the examination of the chest and abdominal cavities, the organs are removed from the body.
The oesophagus is opened along its posterior wall allowing evaluation of oesophagal atresia. The thymus is removed and weighed. The heart is open whilst it is attached to the lungs by the right atrium from inferior vena cava to superior vena cava allowing identification of fossa ovalis and coronary sinus. The right ventricle is open by cutting down the right lateral border of the heart and then along the outflow tract through the pulmonary valve. Tricuspid and pulmonary valves are inspected. The presence and patency of the ductus arteriosus is noted. Left atrium is open between pulmonary veins. The left ventricle is open by cutting down through the mitral valve to the apex and then through the aortic valve into the aorta. Mitral and aortic valves are inspected as the interventricular septum. After heart dissection, larynx and trachea are opened down the posterior wall.
Abdominal organs are then inspected. Gastrointestinal tract is held in order to identify atresia or duplication. Liver, spleen and pancreas are removed. The adrenals, kidneys and ureters are inspected. The concordance between internal and external genitalia is verified. All major organs should be weighed (i.e. thymus, heart, lungs, liver, spleen, kidneys, and adrenals). These weights are compared with standard tables because the weights of the organs vary with age or as a result of a disorder.

Sections of all organs (including thymus, lungs, heart, liver, spleen, pancreas, gastrointestinal tract, kidneys, adrenals, urinal bladder, and internal genitalia) are taken for microscopic examination.

The placenta should be examined in all cases.

In conclusion, a thoughtfully performed perinatal autopsy is an important tool capable of generating valuable information for families, physicians, and society. Understanding the causes of reproductive failure or malformations is important because each death is a personal or family tragedy to avoid repeating if possible.

Selected lectures:

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INVITED LECTURES

L 05
Dermatopathology: Cutaneous Soft Tissue Tumours.

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The spectrum of cutaneous mesenchymal neoplasms includes mainly tumours of nerve sheath, smooth muscle, myofibroblastic, vascular, adipocytic and uncertain differentiation. Their morphological range is wide and includes spindle, epithelioid and pleomorphic cell features as well as overtly
vasoformative or adipocytic elements, giving rise to a large differential diagnosis. While the majority of cutaneous mesenchymal tumours are benign, the differentiating features may be subtle and morphologically bland appearing tumours may be associated with outright malignant behavior. Conversely, tumours showing histological features more commonly associated with malignancy may behave in an entirely benign fashion. Reliable and accurate diagnosis is therefore necessary to predict behavior and guide treatment.
Over the past years significant advances have been made regarding our understanding of the behavior and the molecular genetics of some of these tumours and new entities have emerged:
Atypical Fibroxanthoma (AFX): AFX is a distinctive but controversial entity. Despite histological features of malignancy it has now become clear that the behavior of these tumours is entirely benign if strict criteria are applied. Similar tumours with additional invasion of subcutaneous tissues, tumour necrosis or lymphovascular or perineurial invasion are currently better regarded as pleomorphic dermal sarcoma (PDS) as these features confer at least low-grade malignant potential with risk for local recurrence and even distant metastasis. A common diagnostic problem is the lack of positive discriminatory features and the diagnosis of AFX and PDS is one of exclusion.
Cutaneous angiosarcoma: Rare morphological variants of angiosarcoma have recently been reported. These include foam, signet ring and granular cell change. Accordingly these tumours may be mistaken for a histiocytic or sebaceous neoplasm, signet ring cell carcinoma, atypical lipomatous tumour or even granular cell tumour. Angiosarcoma is rarely suspected morphologically and the final diagnosis is largely dependent on the demonstration of endothelial cell differentiation by immunohistochemistry. The recently reported antibody ERG is highly sensitive and specific and is of particular use in this setting.
Postradiation atypical vascular lesion (AVL): Postradiation AVL and well-differentiated angiosarcoma share many clinical and histological features. Reliable separation is necessary in view of their different behavior and management. However this is often difficult and may be impossible in individual cases. Amplification of the MYC gene has recently been demonstrated in secondary cutaneous angiosarcoma but not in radiation

associated AVL. This can easily be demonstrated by FISH or immunohistochemistry and has become a useful additional test in this diagnostic setting.
Cutaneous nerve sheath tumours: Recently a distinctive group of cutaneous nerve sheath tumours with hybrid features has been reported. The majority of these tumours show a combination of Schwannian and perineurial differentiation. They may represent a diagnostic pitfall. Their behavior is however entirely benign and they are not associated with neurofibromatosis type 1 or 2.
Cutaneous malignant peripheral nerve sheath tumour (MPNST): A commonly encountered problem is the misdiagnosis of desmoplastic melanoma as MPNST. Cutaneous MPNST is an exceedingly rare diagnosis and the diagnosis should be made with caution outside the setting of patients with neurofibromatosis 1 (NF-1).
The majority of MPNST in the skin represent either extension of a deeper seated tumour or malignant transformation of a neurofibroma in the setting of NF-1. In contrast, S100-positive spindle cell tumours on sun-damaged skin of the elderly are better regarded as desmoplastic melanoma.

INVITED LECTURES

L 06
Are you ready for digital pathology?

Xavier Moles Lopez

Abstract
Surgical pathology is entering the digital era, allowing rapid exchanges through expert pathologist networks and large-scale computer-assisted diagnostics. Indeed, considerable improvements in telecommunication technologies as well as the digitization of medical data and images enabled the advent of an increasing number of computer-assisted medical applications, among which, digital pathology.

In the last decade, digital pathology has benefited from the developments of image acquisition enabling the rapid conversion of glass slides into digital images for the microscopic examination of histological or cytological samples and from the worldwide image distribution via the Internet. This niche is rapidly evolving in the pathology domain and its popularity should keep growing at the rate of the technological improvements.

Tomorrow's pathologists will thus work without microscope. Digital pathology fosters efficient communication between general and specialized pathologists. These new communication media facilitate second opinion requests and enable more precise diagnostics for difficult cases, among other advantages. Patients thus benefit from a faster diagnostic, sent to the clinician through a secured web platform. Faster and better diagnostic, and therefore treatment, could reduce health-care costs. In addition, samples from rare cancer cases could be scanned and archived in the Belgian Virtual Tumorbank, which would constitute a gold mine for teaching and research.

The classification of cancers is based on morphology and is supposed to define groups that have similar clinical evolutions. Nowadays, the pathologist is also asked to specifically evaluate each patient's cancer in order to guide the therapeutic choices. This personalized medicine, summarized by the sentence "the right drug to the right patient", requires the development of new diagnostic methods. An increasing number of targeted therapies are made available in association with a companion test able to select patients that will benefit from this therapy.

In the majority of the laboratories, the pathologists perform all these evaluations by eyes, without concordance study. In contrast, the digital slides enable applications in clinical practice of image analysis algorithms, e.g., designed to quantitatively evaluate the expression of a tissue-based biomarker involved in a companion test. Experts agree that image analysis has the potential to improve intra- and inter-observer reproducibility. However, the implementation of image analysis in daily practice remains controversial.

In spite of the technological improvements, choosing and implementing a digital pathology workflow remains a difficult task. Indeed, the solution must adapt itself to the existing workflow of the laboratory and, at the same time, be compatible with the solutions of other laboratories. Therefore, guidelines will be presented to help interested pathologists to define their needs and objectives and to choose an appropriate digital pathology solution.

L 07
Use of digital pathology in quality assessment.

M Vyberg
NordiQC, Institute of Pathology, Aalborg University Hospital, Denmark

While manual quantification of immunohistochemical staining reactions may cause considera-ble inter-reader variability, quantitative digital pathology (QDP) has provided more reliable tools of tissue-based biomarker measurement allowing for standardisation, quality assurance and decision-support measures. In breast cancer, an accurate assessment of HER2, hormone receptors and proliferation markers has met extensive demands with their roles as prog-nostic or predictive markers. QDP algorithms for HER2 expression have shown to ensure more accurate and reproducible results. In a novel HER2 IHC algorithm, developed by Visiopharm in collaboration with NordiQC, a concordance rate with FISH of close to 100% has been shown (1) diminishing the need for confirmatory FISH tests. In external quality assurance this algorithm has furthermore shown to separate optimal from suboptimal HER2 staining reactions. For pa-thology laboratories with optimal staining, the variability was 3% - 14% across different stainer-platforms (which is comparable to gene expression assays), while with suboptimal staining quality, the inter-laboratory variability increased with a factor of 4-6 (2).
With QDP, also standardized quantitative assessment of nuclear markers like estrogen and progesterone receptors and Ki67 can be reliably performed. QDP based mitotic index calcula-tion on phosphohistone H3 stained slides has shown more reliable than mitosis counting based on H&E staining. However, difficulties in defining regions of interest by excluding the stromal compartment may blur the results of standard QDP, and manual outlining of regions of interest is often cumbersome and unreliable. Virtual Double Staining (VDS), also developed by Visio-pharm in collaboration with NordiQC, is intended for automated tumour/stroma separation and computation of e.g., ER and Ki67 labeling index for the identified tumor cells, significantly improving accuracy. Development of VDS algorithms to assess other markers and other neo-plastic lesions are ongoing, leading to of standard testing procedures for a range of tumour markers. The implementation of HER2 QDP and VDS QDP will allow for improved laboratory standardiza-tion and quality assurance, more reliable clinically validated cut-off values, and improved work-flow.

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INVITED LECTURES

L 12
How to diagnose malignancy in well differentiated thyroid tumours?

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1. The prominence of the morphological features for diagnosing malignancy
The diagnosis of malignancy in tumours to be classified as papillary or follicular thyroid carcinoma (PTC and FTC) is almost exclusively based, for the time being, on morphological features. The most important of such features is the demonstration of unequivocal signs of invasiveness (parenchymatous, capsular and/or lympho-vascular invasion). This statement discloses a shift from the idea that the most important criterium for diagnosing malignancy in the large majority of well differentiated thyroid carcinomas (WDTC) is the nuclear features which allow per se the diagnosis of PTC that represents more than 95% of WDTC.
The need to demonstrate capsular and/or lympho-vascular invasion for diagnosing FTC has been established many years ago and kept thereafter. That is the reason why FNA does not allow the diagnosis of FCT.
The same does not apply to PTC in which nuclear morphology became more important than growth pattern (follicular and/or papillary) and was turned eventually into an absolute criterium of malignancy in the diagnosis of PTC. This diagnostic approach has created two major problems: a) How much the nuclear features shall be typical of PTC-nuclei, and how many of such nuclei are needed to make a firm diagnosis of PTC? b) What about non-invasive, follicular patterned tumours composed of cells displaying PTC nuclei? According to the present guidelines they are diagnosed as follicular variant of PTC but are they true cancers?
The first problem has been solved in a subjective way – each one of us uses its own threshold for diagnosing a PTC based upon the nuclear features – and by the creation in difficult cases of a new category of thyroid neoplasms, the so-called well differentiated tumour of uncertain malignant potential (WDT-UMP).
The second problem has started being solved by the division of PTC into classic and follicular variant and, within the latter, into the following subtypes: encapsulated, infiltrative/poorly circumscribed and diffuse/multinodular. This approach has limited the toughest differential diagnostic problems to the encapsulated form of follicular variant since in classic PTC, as well as in infiltrative and multinodular forms of follicular variant of PTC, the diagnosis of malignancy is pretty obvious even when the nuclei are not all, nor fully typical, PTC nuclei. In other words, if one is facing a totally or partially poorly circumscribed, infiltrative thyroid neoplasm the diagnosis of PTC does not depend so much on the nuclear characteristics and we go for it without a major concern. The same does not hold true, however, when one is dealing with an encapsulated tumour (see below).

The importance of invasion for the diagnosis of malignancy is so decisive that whenever one is dealing with an encapsulated thyroid tumour with unequivocal signs of capsular and/or vascular invasion there are three possible diagnoses: FTC, Follicular variant of PTC and Well differentiated thyroid carcinoma, NOS. The latter diagnosis is made when the nuclei of the neoplastic cells are of "intermediate" type, neither typical PTC nuclei, nor common follicular-cell nuclei. For practical purposes it is better to state one is seeing a Well differentiated carcinoma, NOS, than to force its inclusion in any of the two categories (FTC or PTC).

The most important consequence of the above mentioned alterations in the priority of the diagnostic criteria for well differentiated thyroid tumours regards the way encapsulated tumours with PTC nuclei that do not display any signs of invasion are considered. We go on classifying them as Follicular variant of PTC (adding the classification of encapsulated and non-invasive to the diagnosis) but we just recommend a conservative therapy (lobectomy or lobectomy plus isthmectomy). In other words, we include such lesions in the group of carcinomas but we think they should be treated as a follicular adenoma.

The worst problem one is facing in this setting concerns the encapsulated lesions in which there is not vascular invasion but there is unequivocal capsular invasion. These lesions fit in the group of Minimally invasive, non-angionvasive FTC or Encapsulated, minimally invasive, non-angioinvasive follicular variant of PTC and it remains disputable the best way of treating them. I would favour a conservative approach but I am not sure since it is difficult to evaluate the retrospective studies on record and there are no prospective studies available in the literature.

Whenever one is facing an encapsulated tumour without vascular invasion and with equivocal signs of capsular invasion, we go for the so-called "UMP diagnoses": Follicular tumour of uncertain malignant potential (FT-UMP) if the nuclei are of the follicular-cell type, and Well differentiated tumour, UMP (WDT-UMP) if the nuclei are of the "intermediate" type.

All UMP neoplasms should be treated conservatively.

The same criteria – first, invasion features, and afterwards the nuclear characteristics – should be used in the diagnosis of malignancy of Oncocytic (Hürthle cell) tumours. In this setting, the evaluation of nuclear features is even more difficult and therefore the need to search for invasiveness is decisive. In case there are unequivocal of signs of invasion – in oncocytic tumours there is occasionally a multinodular pattern of parenchymatous invasion that fit into the category of "widely invasive" but the mere capsular/vascular invasion is more frequent – one can make three diagnoses according to the nuclear features: Oncocytic variant of PTC, Oncocytic variant of FTC and Oncocytic (Hürtle cell) carcinoma, NOS. The "UMP approach" also applies whenever dealing with encapsulated oncocytic (Hürthle cell) tumours with follicular-cell nuclei in which there are only equivocal signs of capsular invasion: Oncocytic (Hürthle cell) tumour, UMP.

2. The usefulness of clinical and imagiological information for diagnosing malignancy
The most important clinical information goes one being the iodine fixative characteristics of the tumours: a hot nodule is rarely malignant.
The emphasis on the importance of invasiveness for diagnosing malignancy, together with the development of imagiology, will turn progressively more important the imagiological data in the diagnostic approach of thyroid nodules. For the moment, such data are not decisive per se but should always be integrated together with the clinical and the morphological informations (Pathologists are physicians, not more or less glorified technicians)

3. Immunohistochemical and genetic (molecular) data in the diagnosis of malignancy
Immunohistochemistry provides supporting evidence to the diagnosis of PTC. The following immunohistochemical markers can be used: HBME1, galectin 3, cytokeratin 19, fibronectin, sialyl-Lewis a, sialyl Lewis x … In our hands, like in other groups, HBME1 is the most useful. However, neither HBME1 nor any of the other markers provide clear cut evidence in difficult cases, namely follicular variant of PTC. Such biomarkers are very good to confirm unequivocal cases of PTC, namely classic PTC, as well as some variants (e.g. tall cell PTC) but fail in the borderline cases (namely follicular variant of PTC with "intermediate" nuclei, with or without oncocytic features). In our practice we do not use immunohistochemistry for diagnosing PTC. The same holds true regarding the utilization of immunohistochemical markers to identify lympho-vascular invasion in equivocal cases. Either we are able to diagnose such invasion because there are clear images of well preserved neoplastic tissue inside well formed vessels or, if there are not such images, we do not rely on CD31 or CD34 to make the diagnosis.
Assuming that cancer is a successful clone of one of our own tissues that grows without respecting the frontiers – thus emphasizing the importance of invasion and of the epithelium/mesenchymal cross-talk – it seems obvious that there will never be any genetic/epigenetic alteration allowing, per se, the diagnosis of malignancy. This statement holds true in oncology in general and in thyroid cancer in particular.
RET/PTC and PAX8-PPARg rearrangements, as well as RAS mutations, can be found in benign and malignant thyroid tumours and can not therefore be used in the differential diagnosis of controversial cases. BRAF V600E mutation is, in this respect, an exception since it has only been detected in some thyroid carcinomas: PTC, poorly differentiated carcinoma (PDTC) and undifferentiated carcinoma (UC). Without questioning the major pathogenic importance of BRAF mutation in thyroid carcinogenesis, it should be stressed that its putative diagnostic meaning is questionable. It has been demonstrated that the BRAF V600E frequently occurs as a subclonal or oligoclonal event thus introducing complexity in the dual classification (mutated and wild type BRAF) of PTC. It has also been shown that the oncogenic properties of mutated BRAF depend upon its accompanying genetic/epigenetic partners. It is likely that in thyroid, like in melanocytic lesions, the mutated BRAF does not play an oncogenic role when isolated – in nevi, for instance, such mutation induces senescence rather than malignancy. Furthermore, we

start knowing which accompanying partners are needed for inducing a malignant behaviour in thyroid tumours (namely TERT mutation, see below). Anyway (and unfortunately) the BRAF V600E mutation is almost only detected in PTCs that do not raise difficult diagnostic problems (classic PTC, tall cell PTC, Warthin-like PTC,…) and this limits the utilization of its detection as a diagnostic biomarker of malignancy in follicular variant of PTC, as well as in other PTCs with a solid/trabecular growth pattern. We have recently found that mutation of the TERT promoter (leading to activation of telomerase) appears to occur almost exclusively in thyroid carcinomas (8% in PTC, 17% in FTC, 29% in PDTC and 33% in UC). We have also obtained evidence suggesting that TERT mutation plays a prognostic role alone and together with BRAF mutation. These findings have major pathogenic and prognostic meaning but do not seem useful from for diagnosing malignancy.
Besides the specific mitochondrial genetic deletions and mutations that characterize all sorts of oncocytic (Hürthle cell) tumours, we and others found some nuclear genetic alterations that appear to be typical of such tumours. These genetic biomarkers are very important for understanding the etiopathogenesis and the morphological features of the oncocytic phenotype but do not help to separate benign from malignant tumours. The same holds true regarding the analysis of miRNAs that can be linked with oncocytic tumours but are useless to diagnose malignancy in difficult cases. Our assumption that individual genetic markers will not be able to separate benign from malignant tumours applies to miRNAs and long non-coding RNAs (lncRNAs), formerly designated long intergenic non-coding RNAs (lincRNAs); both types of RNAs (very short and lengthy) can be associated with benign or malignant histotypes (for instance, there are miRNAs and lncRNAs which are fairly typical of PTC) but they do not provide evidence to separate borderline cases (As it should be expected if one sticks to the concept that malignancy is related to invasion).

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L 13
Thinprep technique, a single centre experience and review of the literature

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Abstract
Liquid based cytology now plays a significant role in cytopathology. Used historically, in gynaecologic specimens, the technique has gained popularity in non-gynaecologic specimens over the last decade.
In our centre, we use ThinPrep technique for thyroid punctures.
There are several advantages to the use of ThinPrep in thyroid cytology e.g. elimination of blood, optimal cellular preservation and evenly deposited cells on the slide.
On the other hand, ThinPrep technique results in some unique changes in morphology that need consideration in the diagnostic process of thyroid disease e.g. colloid morphology, alternation of the ratio of cell components and less visible intranuclear inclusions.
We will focus on the technique, diagnostic pittfalls and clues when using ThinPrep for thyroid cytology. Especially follicular cell neoplasms will be highlighted, since they still represent a grey area for the cytopathologist (and histopathologist). Briefly we will discuss the role of cell blocks in thyroid cytopathology and application of ancillary techniques in the diagnosis of thyroid diseases such as immunochemistry, flow cytometry and molecular techniques.

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INVITED LECTURES

L 14
Rare tumours of the thyroid.

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This topic will be addressed excluding the numerous variants of papillary thyroid carcinoma (PTC), as well as the rare (and less rare) morphologic variants of medullary thyroid carcinoma (MTC). We will not also discuss the several types of lympho-hematopoietic malignancies that can involve the thyroid, nor the metastatic diseases. With regard to the latter issue (metastases in the thyroid) it is worthwhile stressing that autopsy studies have shown that the gland is frequently involved by microscopic metastatic deposits in many types of generalized cancer, whereas the occurrence of metastases presenting themselves as a primary thyroid tumour is rare. In our experience, such occurrence has been observed mainly in kidney, breast and lung carcinomas. Incidentally, the metastases tend to occur inside preexisting thyroid tumours (follicular adenoma or adenomatous nodule, follicular variant of PTC and MTC) rather than in the normal parenchyma. The co-existence of the two "tumours" (follicular – or C-cell derived host neoplasm and metastatic focus/foci) gives frequently rise to peculiar lesions that may be diagnostically challenging.
To address the aforementioned, complex lesions, as well as to achieve a diagnosis in any thyroid tumour whose diagnosis is not clear (Is it a primary thyroid tumour? If yes, is it a follicular-cell or a C-cell derived carcinoma? Is there room for considering a rare, exceptional situation?), one should always rely on an early immunohistochemical step: To search, systematically, for TTF1, thyroglobulin (Tg) and calcitonin.
There are some rare follicular-cell derived thyroid tumours that are positive for TTF1 and frequently negative for TG: mucoepidermoid carcinoma, diffuse sclerosing variant of PTC and both familial (FAP syndrome) and sporadic form of cribriform-morular carcinoma (CMca). The latter entity is a good example of a tumour that despite being usually classified as a variant of PTC is better described as a well differentiated carcinoma, NOS. Incidentally, rare cases of poorly differentiated carcinoma arising from a CMca, and of CMca with neuroendocrine differentiation have been reported (in both instances the tumours have an aggressive clinical behavior and carry a guarded prognosis).
The utilization of the triple immunohistochemical markers (TTF1, Tg and calcitonin) is also very useful in the diagnosis of columnar cell carcinoma (DD with metastasis of endometrial and colon adenocarcinoma) and well differentiated carcinoma with a glomeruloid pattern of growth. Curiously, CDX2 expression may be detected in the former and WT1 in the latter.

Immunohistochemistry is also crucial for diagnosing the extremely rare calcitonin-negative, well differentiated MTC which is positive for TTF1 and calcitonin-gene related peptide (CGRP). In the differential diagnosis of this peculiar MTC one has to consider, before going for immunohistochemistry, the tentative diagnoses of parathyroid tumour, paraganglioma and metastasis in the thyroid from a clinically occult neuroendocrine carcinoma.
The correct diagnosis of spindle cell tumours of (or in) the thyroid also imply the concurrent use of good old histology and immunohistochemistry. In our experience, most of those tumours fall into the category of spindle cell adenoma, spindle cell variant of PTC or spindle cell variant of MTC; if the tumour is "triple" negative (TTF1, TG and calcitonin) one has to consider other hypotheses such as solitary fibrous tumour (the most frequent spindle cell mesenchymal tumour of the thyroid) and metastasis from a sarcoma.
Whenever the clinical setting (very young patients) and the histological appearance are appropriate the diagnosis of SETTLE (Spindle cell tumour with thymus-like differentiation) should also be considered. The mucinous differentiation may be quite prominent in SETTLE as well as in follicular adenoma, PTC, FTC and MTC. There are also rare mucinous tumours of the thyroid that after excluding a metastasis from a mucin-producing adenocarcinoma, are difficult to classify within the classic categories and should probably be placed, at least for the moment, in the group of well differentiated mucinous tumours of uncertain malignant potential. Some of the aforementioned tumours occur in the setting of Cowden syndrome and other have a striking resemblance with salivary gland tumours.
A last point to emphasize the existence of small cell tumours of the thyroid that do not belong to the most frequent categories (poorly differentiated thyroid carcinoma, small cell variant of MCT and lymphoma). We have been particularly interested in the study of such less frequent or even extremely rare small cell tumours that partially fit into the broad group of PNET/Extraskeletal Ewing Tumours (for a thorough review see the paper by Eloy C et al).

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INVITED LECTURES

L 15
Second opinion in rare thyroid cancers.

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During the last 5 decades, the incidence of thyroid cancer has been rapidly growing worldwide (1). This increase is mainly due to small (≤ 10mm), localized, asymptomatic differentiated thyroid carcinomas (DTC) and a relevant proportion of these cancers are discovered incidentally at surgical pathology, in the absence of preoperative suspicion of malignancy (2).

The tools for DTC diagnosis, treatment and monitoring have enormously evolved over the past two decades and pathology has played a pivotal role in this scenario. In fact, the introduction of Fine-Needle Aspiration cytology (FNAC) in the diagnostic work-up of thyroid nodules on one hand has provided a tremendous contribution to an earlier diagnosis of thyroid cancers (2). On the other hand, an increasingly more precise definition, staging and grading at surgical pathology has proven extremely important for a more effective management of DTCs (3). Thus, in the preoperative setting the choice of the most appropriate treatment mainly relies in most thyroid nodular disease patients on FNAC interpretation. Analogously, in the post-surgical approach to DTCs, the decision to administer adjuvant radioactive iodine ablation, which exposes the patient to potential adverse effects, namely an increased risk for secondary cancer (4, 5) is based on specific elements emerging from pathology. For these reasons, the availability of an expert pathologist is crucial in order to maximize the diagnostic accuracy of thyroid nodule evaluation or to plan adequate management programs of thyroid cancer patients. Moreover, a second reading and/or analysis with ancillary techniques for either cytological specimens in the preoperative setting or histological preparations in the post-surgical setting may in specific instances be advised.

In a recent document the *Belgian Health Care Knowledge Center* (KCE) has identified several rare or complex cancers that should be treated in reference centers, fulfilling some specific requirements in terms of minimal volumes of patients and organization of care (6). Within this framework, a group of Pathologists were asked by the KCE to release the recommendations for obtaining accurate cancer diagnosis, also through a second opinion in Pathology, in all those conditions presenting high diversity and complexity of lesions, or requiring sub-specialization associated with the application of ancillary techniques.

DTCs have been included by the KCE among those cancers requiring Reference center management. This presentation will illustrate some circumstances in which a second

opinion or the use of ancillary techniques may improve either the diagnosis or the management of DTCs.

Pre-operative setting

During the last 2 decades, the widespread use of thyroid FNAC and the diffusion of standardized reporting methods provided an important contribution to an improved definition of cancer risk for each FNAC diagnostic category (3). Nevertheless, the "indeterminate" category of thyroid neoplasms still represents a major challenge for both the pathologist and the clinician, because of the impossibility to provide a conclusive pre-operative diagnosis, irrespective of the employed classification system used for cytology reporting. Histological verification as the "gold standard" is usually required in such instances, leading to the identification of benign lesions in most patients (3), who eventually undergo "diagnostic" thyroidectomy. Moreover, the issue of variability in thyroid cytology reporting is being recognized as an emerging difficulty, particularly in "indeterminate" thyroid lesions. Some preliminary studies performed in USA after the adoption of the Bethesda System have shown a rather wide variation in inter-observer agreement rate among cytologically indeterminate categories (7). More surprisingly, another study reported also an intra-observer discordance rate of 25% for blinded second readings (8).

Ancillary techniques could be of help in overcoming such a difficulty. In tis respect, however, the contribution of immunocytology resulted rather disappointing. In fact, the use of markers such as galectin-3 (9-13), HBME-1(10, 11, 13), fibronectin, cytokeratin-19 (10, 11) showed limited diagnostic accuracy with prominent overlap between follicular adenoma and differentiated thyroid carcinomas (14, 15).

More recently, the identification of different oncogene mutations occurring in DTCs opened new perspectives for the classification and diagnosis of thyroid tumors, suggesting molecular analysis as a complement to histology. The search of several somatic mutations has become increasingly used and seems the most promising future approach for molecular FNAC diagnosis (16-18). At present, however, no single applied marker presents the accuracy and precision of histopathologic diagnosis. Recently, several attempts have been made to validate the suitability of molecular analysis by using panels of gene expression profiles able to identify malignant thyroid lesions, with promising results (19- 21).

Second reading in the post-operative setting

Accurate risk stratification of DTC patients is considered essential for optimal treatment and improved follow-up (3, 22-23). As for other malignancies, the first step in DTC post-surgical management is an accurate staging, which relies on the system elaborated by the American Joint Cancer Committee/Union Internationale Contre le Cancer (AJCC/UICC). This staging system is based on four variables: patient age, the size and

extension of the primary tumor (pT), lymph node involvement (N), the presence of distant metastases (M) and allows to classify DTCs according to the probability of recurrence. Currently available guidelines from both the European and American Thyroid Associations (ETA, ATA) define 3 levels of risk: high, intermediate, or low, on the basis of pTNM staging and other parameters available shortly after initial surgery (3, 22-23). Low risk DTCs include: classical variant of papillary thyroid cancer (PTC) T1-3N0M0 and the so-called "minimally invasive" follicular thyroid carcinoma (FTC). Approximately 15-25% of DTC have an intermediate risk of recurrence. This class of DTCs includes: T1-3N1M0 classical Papillary thyroid carcinomas (PTC), all T1-3N0-1M0 aggressive variants of PTC (insular, tall cell, trabecular, Hurtle cell, diffuse sclerosing), T1-3N0-1M0 follicular thyroid carcinoma, Hürtle cell carcinoma (3, 22-23). Only a minor proportion (5-10%) of DTCs carry a high risk of recurrence. The high risk is defined by presence of distant metastases, incomplete tumor resection, macroscopic invasion of peri-thyroid soft tissues, serum Thyroglobulin (Tg) out of proportion as compared to post-treatment scan (3, 22-23). It is, therefore, evident that important elements emerging from surgical pathology play a pivotal role for assessing prognostic factors and may influence management of DTC patients, with consequent implications on outcome. As an example, we may recall that the attribution to low or intermediate class risk for similar pTNM staging solely relies on tumor histotype. Thus, some histologic subtypes (i.e. tall cell, columnar, insular, and solid variants), as well as poorly differentiated thyroid cancer are considered worrisome (3, 22-23). Analogously, invasion of intrathyroidal vasculature, multifocal disease (gross or microscopic); and non-papillary histology (i.e. follicular thyroid cancer and Hürthle cell cancer with vascular invasion) are considered at higher risk (24-27). Conversely, the so-called minimally invasive follicular thyroid cancers, which are characterized histologically by tumor capsule penetration without vascular invasion, are generally regarded as lower risk tumors, irrespective of the tumor size (24-28).

Because all non-classical DTC histotypes are rare, their exact definition relies on the experience of the pathologist. In addition, the assessment of the above mentioned prognostic and predictive factors is prone to errors and needs strict quality control. In order to improve patient management, a second reading or the use of ancillary techniques could be advised for such cases.

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L 16
Application of next generation sequencing to fine needle aspiration.

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The assessment of thyroid nodules is a common clinical problem. Thyroid fine needle aspiration (FNA) is the standard preoperative tool for diagnosis of thyroid nodules. However, the limitation of this procedure consists in the high rate of indeterminate or suspicious cytological diagnosis which leads to unnecessary surgical intervention.

Recent progress in understanding the molecular pathogenesis of thyroid cancer has lead to the identification of driver mutations and biomarkers of thyroid cancer. This has mainly resulted from the identification of molecular alterations of signalling pathways, such as RAS-RAF-MEK-MAPK pathway as well as gene translocations such as RET–PTC or PAX8–PPARG.

All these genetic alterations in thyroid cancer have prompted the search of somatic mutations in material obtained by FNA in order to increase the diagnostic accuracy of cytology. Several prospective studies have shown that testing for mutation in BRAF and RAS genes as well as the detection of gene rearrangements is feasible on FNA and provides helpful diagnostic information. The revised American Thyroid Association's management guidelines recommend now to consider molecular testing for these markers for nodules with indeterminate FNA cytology. However, these molecular tests are not yet largely applied in the current daily practice in Europe. This can be explained by the fact that the number of markers to test is high and that the sequential analysis of all these markers is too expensive and time-consuming.

Recently, a new approach, i.e. next generation sequencing (NGS) has begun to supplant other technologies for gene sequencing. This technology enables the simultaneous sequencing of millions of short DNA sequences starting from a very small amount of DNA, and offers benefits relating to lower costs, increased workflow speed and enhanced sensitivity in mutation detection. Many studies have demonstrated the feasibility and benefits of NGS evaluation in various cancer types such as lung, colon or thyroid cancer.

The use of NGS on FNA samples might thus enable a great improvement of cancer diagnosis in thyroid nodules.

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L 23
Update in inflammatory disorders of the oesophagus.

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Inflammatory lesions of the oesophagus are common and they can result from various causes. However, gastro-oesophageal reflux disease (GORD) is by far the most common cause of oesophagitis, and is indeed one of the most common pathological conditions in humans, affecting up to 40% of people in the western world. Other aetiologies of oesophagitis include infections, toxicity of ingested substances, direct traumatic lesions, and oesophageal involvement by systemic diseases. Most of these various forms of oesophagitis can therefore be considered as rare diseases (1,2).

The aim of this presentation is to review the place of pathological study of biopsies in the diagnosis of oesophagitis, with an emphasis on recent changes in practice and on description of new entities.

Classically, histology in reflux disease is considered as having low diagnostic sensitivity and specificity. Most clinical guidelines consider that there is no role for endoscopic biopsies in the diagnosis of GORD, except when it is complicated by Barrett's oesophagus, or in cases with atypical endoscopic features that make necessary to exclude other causes of oesophagitis (3). However, this assumption is based mostly on relatively ancient series of patients, with low value biopsies taken in a non-standardized way, and with seminal histological criteria described more than 40 years ago (4,5). There has been a recent renewal of interest in the description of precise histological signs of GORD, incorporating new features such as dilation of intercellular spaces, total epithelial thickness, carditis, presence of a multilayered epithelium, etc… (6-12). The "Esohisto" project has convincingly demonstrated that these signs are recognizable and reproducible, and as confirmed by other series, it has shown that they have good diagnostic performances to distinguish non-erosive reflux disease (NERD) from functional heartburn, a major clinical issue. Interestingly, some new endoscopic techniques that allow to obtain microscopic images without taking biopsies may help in the diagnosis of NERD, as it was suggested for confocal Laser endomicroscopy (13).

Among other types of oesophagitis, an important event has been the recognition of eosinophilic oesophagitis as a relatively frequent cause of "non-reflux, non-infective" inflammatory oesophageal disorder. Although only relatively recently individualized, eosinophilic oesophagitis is the second most common non-neoplastic oesophageal condition that may require endoscopic examination in both children and adults, after reflux oesophagitis. Its diagnostic criteria have been well established, and require the

presence of symptoms of oesophageal dysfunction, with at least 15 eosinophils / HPF in oesophageal biopsies, after exclusion of competing causes of oesophageal eosinophila (14). Although there are still a number of unresolved question concerning eosinophilic oesophagitis, including pathophysiological, diagnostic and therapeutic issues (15), the most important recent novelty in this field is the recognition of proton pump inhibitor – responsive oesophageal eosinophila ("PPI-REE") as a separate entity (16).
In other types of oesophagitis, there have been no major new data regarding various types of infective and toxic oesophagitis. The only recent relevant publications concern "sloughing" oesophagitis, a rare and severe form of oesophagitis (17), and lymphocytic oesophagitis, a recently identified form of oesophageal inflammatory disorder, with a purely histological diagnosis, and with poorly recognized etiological and clinical characteristics (18).

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L 26
Inflammatory diseases of the colon.

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Introduction

The term chronic inflammatory bowel disease (CIBD) infers ulcerative colitis, Crohn's disease and the spectrum of pathology that lies between, encompassing indeterminate colitis and other cases of equivocal inflammatory bowel disease. There are an inordinate number of conditions, many of which bear the title 'colitis' which may show strong pathological mimicry of idiopathic chronic inflammatory bowel disease and may be included under the umbrella term 'inflammatory bowel disease' (1,2). Through the lecture, it will become increasingly clear to the interested listener that **context** is a critical determinant of the pathological diagnosis in inflamed biopsies from the colorectum and is crucial in differentiating true CIBD from its many mimics.

The lecture will focus on infective colitis, drug-induced colitis, diverticular colitis, focal active colitis, microscopic colitis and secondary colitis. All these conditions show pathological features similar to those seen in CIBD. There are also interesting parallels and associations between these conditions and CIBD. A small proportion of diverticular colitis cases will eventually re-present with classical ulcerative colitis, despite, initially, a normal rectal histology. In a similar proportion of cases, focal active colitis precedes the onset of classical Crohn's disease. Drugs, especially NSAIDs, have an extraordinary relationship with CIBD. They cause (?), they mimic, they exacerbate, they induce and they treat CIBD.

The diligent pathologist must be aware of the important mimics of CIBD and ensure that they are fully cognisant with the clinical, endoscopic, microbiological and radiological context before committing a patient to a definitive diagnosis of CIBD. In this hand-out the author will concentrate on two of the more important mimics, infective colitis and diverticular colitis.

Infective colitis mimicking CIBD

The differential diagnosis of an early acute 'colitis' may present considerable difficulties. There are, however, several histological features that may indicate a diagnosis of an acute self-limiting (i.e. infective) colitis or alternatively chronic inflammatory bowel disease (CIBD), even in the absence of well-established diagnostic features of either condition. [1] Crypt architectural distortion takes about 6 weeks to develop in CIBD. [2,3]

Diffuse crypt architectural distortion is a strong pointer towards CIBD, particularly ulcerative colitis as is a villiform surface architecture: these architectural changes are most unusual in infective colitis, being effectively never seen in acute infective colitis and only rarely seen in chronic colitides such as shigellosis and occasionally chronic amoebiasis. [4] The presence of diffuse chronic inflammation is a useful marker of CIBD, although this may be occasionally seen in some infective diseases. [5] Basal lymphoid aggregates are also a good histological pointer to CIBD, in particular ulcerative colitis. [4] Mucin depletion is a variable feature of chronic inflammatory bowel disease: mucin preservation is seen more in Crohn's disease than ulcerative colitis, although this is not an entirely consistent and reliable sign. [6,7]

Acute inflammation is usually diffuse in nature in ulcerative colitis and predominantly involves the epithelium. However, in Crohn's disease and some infections, the polymorph infiltrate may be distinctly patchy and is often more pronounced in the lamina propria than in the epithelium. [8,9] Occasionally the classical histological features of an acute self-limiting (infective) colitis may be present: these include normal crypt architecture, gross lamina propria oedema and focal collections of neutrophils within the lamina propria with some ingress into the epithelium. [1,2,3,4] Crypt abscesses may also be seen and are often eccentric or demonstrate a classical pattern of crypt beading. [1,2,3,4] However such features are not always seen and some cases of infectious colitis are good mimics of early CIBD. Campylobacter jejuni colitis characteristically shows mimicry of CIBD histologically, especially if there is some chronicity to the infection. [3] Amoebic colitis may also be a difficult differential diagnosis. However, if the characteristic haematoxyphilic necrotic slough on the surface of an ulcer is found within the biopsy, it is a good indication for this diagnosis, although one may have to search carefully to see amoebae which have a prominent nuclear karyosome and have incorporated red blood cells into their cytoplasm. [10] There may be difficulty in differentiating amoebae from macrophages containing red blood cells but here the nuclear detail and size of the cell are useful, as are a periodic acid- Schiff (PAS) stain (which will highlight the amoebae) and an immunohistochemical stain for macrophages (such as CD68).

Diverticular disease and its mimicry of CIBD

In former years, it was generally believed that most, if not all, of the inflammatory complications of diverticular disease occurred exclusively in or around the diverticula themselves and that the luminal mucosa of the sigmoid colon showed little or no active inflammation. In a personal study of sigmoid colonic resection specimens, this was shown to be very much not the case: indeed in about 25% of such sigmoid colons, there are inflammatory changes in the luminal mucosa. [11] However, it is manifest that such luminal inflammation is usually asymptomatic or that the symptoms associated with other complications of diverticular disease far outweigh those of this luminal inflammation. Whilst such pathology is now well described, [11,12] the relative paucity of

literature cases seemingly attests to its apparent lack of importance in producing symptoms, compared with other complications of diverticular disease. However this complication of diverticular disease is increasingly recognised as an important mimic of chronic inflammatory bowel disease and one that can provide considerable perplexity to the diagnostic pathologist. [12]

Diverticular colitis

In elderly patients with diverticular disease, endoscopists relatively commonly see mucosal hyperaemia, oedema and erosions, especially on the crescentic folds of the sigmoid colonic mucosa, often well away from the diverticular orifices themselves. [13,14,15] In symptomatic cases of such luminal inflammation, the presentation is characteristically with rectal haemorrhage and/or diarrhoea and the passage of mucus. [13,14] Pathologically such changes represent a veritable profligacy of pathological changes, varying from mild inflammation with oedema and telangiectasia through mucosal prolapse changes to florid chronic active inflammatory changes closely mimicking chronic inflammatory bowel disease. [12,13,16,17,18] Not only is the pathology highly variable, but the extent of the inflammation, the clinical context and the ultimate significance of the changes are also notably capricious.

Various appellations have been applied to this condition, including diverticular colitis, crescentic colitis, segmental colitis, sigmoid colitis and sigmoiditis. [11] Segmental colitis is the preferred term in some quarters [14,16] but this author believes that this term is neither specific nor descriptive enough and that diverticular colitis is to be strongly preferred. Curiously the condition may actually occur before the diverticula themselves are present, in so-called pre-diverticular disease, suggesting that it is not the presence of the diverticula that is important for the genesis of these inflammatory changes but the pre-existing intraluminal and intramural pathological factors that presage the development of the diverticula. [11,19] The pathogenesis of diverticular colitis is probably multifactorial. [11]

In the most severe forms of the disease, where there is marked chronic active inflammation, there may be ulceration, diffuse and marked chronic inflammation, crypt abscess formation and crypt architectural distortion. These microscopic features strongly mimic chronic inflammatory bowel disease of the ulcerative colitis type. [12,13,17,18] Indeed it is now well described that this form of diverticular colitis will respond to medications similar to that used in chronic inflammatory bowel disease. [12,13,14,17,20] Intriguingly, some cases of such diverticular colitis, initially strictly limited to the sigmoid colon affected by diverticular disease, have subsequently evolved into classical distal chronic ulcerative colitis. Initially the rectal mucosa has been entirely normal and yet, subsequently, it has demonstrated all the characteristic endoscopic and histopathological features of ulcerative colitis. It has been postulated that the diverticulosis provides the milieu, possibly by inducing faecal stasis and bacterial mucolysis, that enables classical chronic ulcerative colitis to subsequently supervene. [11,12] Whatever the significance of this

intriguing association, it is clear that this phenomenon is unusual and it remains possible that the association is not causal but merely fortuitous.

In summary, diverticular colitis represents a pathologically intriguing spectrum of disease, often asymptomatic (seemingly), that may closely mimic chronic inflammatory bowel disease and providing a dangerous trap for the unwary pathologist and gastroenterologist, who may make an inappropriate diagnosis of chronic inflammatory bowel disease. However, in a few cases, the disease is very much associated with chronic inflammatory bowel disease of the ulcerative colitis-type.

Diverticulosis and chronic inflammatory bowel disease

Diverticular disease and inflammatory bowel disease are both common conditions of the elderly in Western populations. It is therefore to be expected that these conditions will coexist purely by chance, unless one protects from the other. The latter is clearly not the case: in fact, there is some evidence of an increased co-existence of the two conditions, especially in elderly women, although it is likely that the literature tends to overcall the association. [12,21] For instance diverticular colitis (vide supra) is an important mimic of ulcerative colitis, related, in part, to similar pathogenetic mechanisms, perhaps most notably faecal stasis.

In more than 50% of elderly patients with Crohn's disease, there is concomitant diverticular disease. [22] The literature would suggest that Crohn's disease may preferentially involve the colon affected by diverticular disease and may induce diverticulitis in patients with diverticular disease. [23,24] The co-existence of Crohn's disease and diverticulitis seems to carry a worse prognosis, with a more complicated clinical course than in either disease in isolation. [25,26] However it is important to realise that complicated diverticular disease itself may induce pathological changes that are virtually indistinguishable from classical Crohn's disease affecting the sigmoid colon. [21,27,28] All three of the diagnostic hallmarks of Crohn's disease, transmural inflammation, fissuring/fistula formation and granulomata, may be caused by diverticular disease and diverticulitis. [21,27] Granulomatous cryptitis, thought to be a characteristic feature of Crohn's disease, is often found in diverticular colitis. [11] Even granulomatous vasculitis and regional lymph node granulomata have been demonstrated in complicated diverticular disease alone. [29]

Many older patients have coexistent atheromatous disease, leading to relative ischaemia of the colon. The combination of this and changes in bacterial flora, secondary to stasis within diverticula, is thought to be in part responsible for the Crohn's-like inflammatory picture in the sigmoid colon affected by diverticular disease. [12,21] Three major retrospective reviews strongly support the view that most cases of putative dual diagnoses in the sigmoid colon, namely Crohn's disease and diverticulitis, merely

represent complicated diverticular disease alone. [21,28,29] In all three of these surveys, the great majority of cases showed no positive evidence of Crohn's disease, either at review of the original pathology or on subsequent follow-up. It is notable that the outcome of patients with perceived dual pathology is usually remarkably good following resection, with many patients showing no evidence of recurrence of 'Crohn's disease' elsewhere. [15] This strongly suggests that many of the reported cases do not represent true co-existent Crohn's disease, but rather represent an idiosyncratic inflammatory response to the diverticular disease alone. [15,21]

Whilst this author does not deny that Crohn's disease and diverticulosis can co-exist and that their coincidence may worsen the prognosis of either disease, caution is still required before making the pathological diagnosis of coexistent disease, in the absence of collateral evidence to support a diagnosis of Crohn's disease. Certainly all the features of Crohn's disease may be seen in endoscopic biopsies from complicated diverticulosis and, as already indicated, resection specimens of diverticular disease may show pronounced mimicry of Crohn's disease. Radiological assessment, notably barium enema, is said to be very useful, if dual pathology is suspected, by demonstrating transmural fissuring and extraluminal longitudinal sinus tracts characteristic of Crohn's disease, [23] although one could argue, again, that these may also be seen in complicated diverticular disease alone. Endoscopy may show aphthous ulcers but this is not specific as they may be found in the presence of mucosal inflammation associated with diverticular colitis. [17] Perhaps only if there is evidence of Crohn's disease elsewhere in the intestines should a dual diagnosis be definitively made.

Both diverticular disease and ulcerative colitis are common in the elderly and co-existence is well described. Nevertheless ulcerative colitis and diverticular colitis bear a close resemblance to each other histologically. In most cases, a rectal biopsy is needed to differentiate between these two conditions. However, as previously discussed, there are now several reports of the progression from diverticular colitis to otherwise classical ulcerative colitis (vide supra). As with Crohn's disease, evidence for the dual diagnosis of ulcerative colitis and diverticular disease should be sought elsewhere, principally in the rectal mucosa, before the joint diagnosis can be securely made.

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L 27
Update on gastric cancer with an emphasis on molecular pathogenesis.

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Most gastric carcinomas (GC) are sporadic (90%), familial clustering occurs in about 10% of the cases and 1%-3% are hereditary.

Sporadic gastric cancer

GC is the result of accumulated genomic damage affecting cellular functions essential for cancer development. Three phenotypes of instability have been identified in GC: microsatellite instability (MSI), chromosomal instability (CIN) and a CpG island methylator phenotype (CIMP).
Microsatellite instability (MSI) – The deficiency or inactivation of one of the DNA mismatch repair proteins MLH1, MSH2, MSH6, or PMS2 impairs the repair of naturally occurring DNA replication errors, leading to the appearance of new alleles not present in the normal DNA – the so-called 'MSI phenotype. MSI can cause subsequent genetic changes in numerous genes which have also been demonstrated in GC. MSI has been reported in 15 to 38% of GCs and is mainly caused by epigenetic silencing (promoter methylation) of the *MLH1 gene*. Gastric carcinomas with a high level of MSI (MSI-high) are characterized by antral location, intestinal phenotype, expanding growth pattern and favorable prognosis when compared with tumors with MMR proficient tumors.
Chromosomal instability (CIN) – About 80% of sporadic GCs show CIN, characterized by numerical (gains, losses, and amplifications) or structural changes (e.g. translocations), with aneuploidy.
CpG islands methylator phenotype (CIMP) – CIMP is characterized by hypermethylation of gene promoters leading to gene silencing. Much overlap between MSI and CIMP has been observed in GC. CIMP phenotype has been shown to be associated with widespread hypermethylation, young patient age, and adverse patient outcome in a disease stage independent manner.
Furthermore, molecular alterations can affect specific genes involved in the pathogenesis of GC.
Oncogenes – Some oncogenes are altered preferentially in diffuse carcinoma, such as *BCL2* and *FGFR2* (formerly *K-SAM*). Other oncogenes are altered both in intestinal and diffuse carcinomas, including *CTNNB1* (encoding beta-catenin), *MET* and *MYC*, and *HER-2* and *KRAS* are preferentially altered in intestinal-type.
HER-2 amplification or overexpression has been reported in 7–34 % of the tumors. The currently accepted protocol to detect HER-2 aberrations encloses the use of IHC with specific antibodies and in situ hybridization techniques (FISH, CISH and SISH).

Those cancers scoring 3+ by IHC are considered HER2 positive; those scoring 2+ undergo further confirmation by FISH, CISH or SISH; and those scoring <2+ by IHC are considered HER2-negative cases. There is evidence that HER-2 positive GC may respond well to therapy with the humanized monoclonal antibody trastuzumab, as shown in ToGA trial.
Tumor suppressor genes – Many tumor suppressor genes have been implicated in GC, including APC and DCC in intestinal-type carcinomas, as well as CDH1 and RB1 in diffuse carcinomas. Other tumor suppressor genes are altered in both types of GC, such as PTEN and TP53, though the latter are more common in intestinal-type carcinoma.

Whole genome studies
Modern high-throughput molecular methods are being used with the aim to contribute to a better understanding of the biology of GC at a molecular level.
Two recent genome-wide copy number profiling studies revealed systematic patterns of molecular exclusivity, and genes related to RTK/RAS signaling (*FGFR2, KRAS, HER-2, EGFR* and *MET*) were shown to be frequently amplified in GC in a mutually exclusive manner. These results suggest the existence of five distinct GC patient subgroups, defined by the signature genomic alterations of *FGFR2, KRAS, EGFR, HER-2* and *MET*. Collectively, these subgroups suggest that at least 37% of GC patients may be potentially treatable by RTK/RAS directed therapies.
By exome sequencing, it was found an average of 50 mutations/ GC, mostly affecting genes involved in cell adhesion and chromatin remodeling, and identified two new putative tumor suppressor genes, FAT4 and ARID1A.
Recently, gene expression profiling using mRNA consensus clustering has revealed three distinct GC subtypes – mesenchymal, proliferative and metabolic, with different responses to treatment (the results of preclinical studies suggest that mesenchymal-subtype GCs may be more sensitive to PIK3CA/mTOR/AKT pathway targeting drugs compared with GCs of other subtypes).

Hereditary gastric cancer
Hereditary GC contributes to 1%-3% of the burden of stomach cancer. Two syndromes have been identified: Hereditary Diffuse Gastric Cancer (HDGC) and Gastric Adenocarcinoma and Proximal Polyposis of the Stomach (GAPPS).
Hereditary Diffuse Gastric Cancer – HDGC is an autosomal dominant cancer susceptibility syndrome characterized by signet ring cell (diffuse) GC and lobular breast cancer. In clinically defined HDGC, *CDH1* mutations are detected in 30-40% of cases. Most (75 to 80%) are truncating mutations and the remaining are missense mutations. In addition to point mutations, large germline deletions have been found in 5% of HDGC families that tested negatively for point mutations. In HDGC, the *CDH1* wild allele is inactivated, most frequently by promoter hypermethylation (epigenetic modification) and, less frequently, by loss of heterozygosity (LOH) and CDH1 mutations. Intragenic deletions in the wild-type allele have also been reported.

Germline mutations in TP53 gene were detected in two studies of families with GC and no mutation in *CDH1*. Recently, α-E-catenin (CTNNA1) mutations were identified in HDGC families.

Gastric Adenocarcinoma and Proximal Polyposis of the Stomach – In 2011, a new hereditary gastric cancer syndrome was identified: "Gastric adenocarcinoma and proximal polyposis of the stomach" (GAPPS). GAPPS is a unique gastric polyposis syndrome with a significant risk of gastric adenocarcinoma, characterized by the autosomal dominant transmission of fundic gland polyposis (FGP), with areas of dysplasia or intestinal type GC, restricted to the proximal stomach, with no evidence of colorectal or duodenal polyposis. More recently, two Japanese families were reported displaying the same clinicopathological features.

The syndrome is characterized by incomplete penetrance, with a few elderly obligate carriers having normal endoscopies. Mutations in *APC, MUTYH, CDH1, SMAD4, BMPR1A, STK11* and *PTEN* were excluded in several GAPPS families. Causal genetic defect remains unidentified.

INVITED LECTURES

L 30
The genesis pancreatic tumors.

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The exact morphological classification of tumors of the pancreas combined with their molecular analysis has broadened our understanding of cancer development and progression in the pancreas. It is evident from these studies that the general phenotypical classification of pancreatic neoplasms into tumors with either ductal, acinar, endocrine, or indeterminate differentiation is associated with distinct molecular profiles that suggest that there are profound differences in the molecular pathways that lead to the various types of pancreatic neoplasms. The molecular pathway of neoplasms with ductal differentiation is characterized by K-ras mutations, which seem to mark the first step in the development of these cancers. The second step then includes alterations in the tumor suppressor genes p16, p53 and DPC4. In cystic neoplasms, there are type-specific genes mutated such as GNAS, RNF43 and VHL. Tumors with acinar, endocrine, or indeterminate differentiation, in contrast to ductal tumors, follow molecular pathways that are not initiated by a K-ras mutation. Instead, there is involvement of the APC/β-catenin pathway, as in acinar cell carcinoma and pancreatoblastoma, the MEN1, DAXX/ATRX, HIF-α and Glucagon-receptor pathways, as in neuroendocrine tumors, and the pure β-catenin pathway, as in solid-pseudopapillary tumors. The fact that the various molecular pathways are associated with certain phenotypes of the pancreatic neoplasms suggests that the stem cells from which the tumors originate are already more or less committed to distinct differentiation pathways. Recognition of the type-specific pathways opens avenues for targeted treatment.

L 31
Update in dysplasia of the oesophagus.

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Oesophageal cancer remains a public health problem in most countries, featuring among the 5 to 10 most frequent cancer killers in several parts of the world. However, there have been important epidemiological changes for some years, with a major increase in adenocarcinoma, especially in the Western world, and a relative decrease in squamous cell carcinoma (SCC). A morphological sequence of neoplastic transformation is well described in both types of cancer, with a preneoplastic state preceding invasive cancer. For adenocarcinoma, this preinvasive stage develops in almost all cases in Barrett's oesophagus, an acquired columnar metaplasia resulting from gastro-oesophageal reflux disease. In squamous mucosa, the sequence of events seems to consist of basal cell hyperplasia, dysplasia, and carcinoma. But regarding SCC, this sequence has been described mainly in major high-risk populations, especially in Iran, China, and South Africa, and is less well documented in Western countries where SCC develops mostly in individuals with excess in alcohol and tobacco consumption.

When dealing with an "update" in oesophageal dysplasia, it is difficult to consider equally adenocarcinoma and SCC. Probably because of these major epidemiological differences, with one disease (adenocarcinoma) affecting white adults (mostly men) in Western countries with a rapidly increasing incidence, while the other disease (SCC) develops in high-risk populations far away from North America and Europe, the amount of original data published in these fields strikingly differs. As an example, on Pubmed, with as key words "esophagus & dysplasia", among 131 articles published in 2014, 120 concern adenocarcinoma (and in most cases Barrett's oesophagus), 3 both types of tumours, and only 8 squamous lesions. However, it is somewhat paradoxical that most publications concern a disease with a risk of cancer that may have been overestimated in the past (1), and with unproven efficacy of surveillance programs in term of public health.

Therefore, the main points that can be discussed when reviewing novelties in oesophageal dysplasia are in majority devoted to Barrett's neoplastic transformation, and are the followings:

- The recent publication of updated guidelines on the diagnosis, surveillance, and treatment of Barrett's oesophagus, dysplasia, and carcinoma, originating from the USA and the UK (2,3).
- he persistence of some ambiguity in the terminology to be used to describe

these lesions. This problem is obvious when reading the 4th WHO classification of tumours of the oesophagus, using the same terms but not with the same priority rating for the two types of mucosa: intraepithelial neoplasia (dysplasia) for squamous lesions, and dysplasia (intraepithelial neoplasia) for glandular lesions. It is really time to adopt the same terminology for the entire GI tract, which could be the case in the next 5th WHO classification?

- The description of "new forms" of dysplasia in Barrett's mucosa, distinct from the classical intestinal form (4). A serrated subtype exists, very uncommon, and that can be added to the list of serrated precancerous lesions in the GI tract. More importantly, the recognition of a "foveolar" subtype has to be known by all pathologists in charge of biopsies from Barrett's mucosa, as this subtype is clearly more difficult to recognize and to classify (5). The recent description of the "deep or cryptic" type of dysplasia in Barrett mucosa is also important, as it contradicts the previous "dogma" of a constant surface involvement in glandular dysplasia in the GI tract (6). This type is also more difficult to recognize, and its significance in terms of cancer risk remains to be determined.
- The grading of dysplasia may still appear as an incompletely answered question, both in Barrett mucosa and in squamous epithelium. In Barrett mucosa, the three-tiered system (mild / moderate / severe) is now almost universally replaced by a two-tiered system (low-grade / high grade). However, the place and significance of the category "indefinite for dysplasia" remains a matter of debate. On the other hand, the two-tiered classification may even be too complicate and with less and less clinical relevance; in a recently published excellent overview on the role of pathologists in Barrett, Hopcroft and Shepherd suggest that this classification might soon be simplified very much (7). They argue that the therapeutic consequences are more and more similar for low-grade and high-grade dysplasia, due to the development of non-surgical procedures, and therefore they predict that the next classification could be a "one-tiered" system, non-dysplastic vs. dysplastic mucosa.
- The situation remains ambiguous for squamous dysplasia. Although the WHO classifies this lesion as high-grade or low-grade, some textbooks and several articles still use a mild / moderate / severe dysplasia or intraepithelial neoplasia grade 1/2/3 system, similar to that in use in many other organs with squamous precancers and cancers. The recent proposal of a unified two-tiered classification for lower anogenital squamous precancerous lesions may inspire experts in oesophageal squamous lesions, as this two-tiered system is obviously more reproducible and more easy to use in terms of therapeutic decisions (8).
- For both types of mucosa, although there has not been any recent work assessing this point, it is certain that the major differences between "Western style" and "Asian style" pathologists in the diagnosis and grading of early neoplastic lesions of the oesophagus remains, despite the publication of the Vienna classification, the main object of which was to unify these divergent opinions.
- Regarding the biomarkers that can be used in complement to standard

histology, nothing really new has appeared, despite numerous publications. The only marker that can be used in routine practice is p53 immunohistochemistry, of special interest to distinguish among patients with low-grade dysplasia in Barrett those at risk of a rapid evolution to high-grade lesions (9). Interestingly, the recognition of an "absent" pattern of p53 expression improves the sensitivity of this marker, and may even delineate a subpopulation with an especially high risk of adverse outcome (10).

- Indeed, a major critical point in Barrett is to better know the individual risk of evolution to high-grade lesions in patients with low-grade dysplasia. There have been various studies published on this subject, with still diverging results, which might be due in part to the poor diagnostic reproducibility of this diagnosis, still a problem in 2014 (11-13). The rapid development of virtual slides resulting in a much easier access to expert diagnostic confirmation may improve this reproducibility.

Although they are not in use in routine practice at the present time, new technologies will clearly offer very soon great possibilities of improvement in basic knowledge and diagnostic and therapeutic procedures in oesophageal precancerous lesions. Two examples can be given. In the "fundamental" research field, it is now possible to use the "NGS" technology to better define preneoplastic sequences of events in Barrett transformation (14,15). From a clinical point-of-view, clinicians are now able to use techniques that allow endoscopical "microscopy", with so-called virtual biopsies, with at the first place confocal laser endomicroscopy. These techniques will modify very much the current practice of GI pathology in precancerous lesions of the oesophagus (and also in the rest of the GI tract) in the near future.

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L 32
THE NON-NEOPLASTIC ENDOMETRIAL BIOPSY.

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Although the non-neoplastic endometrial biopsy lacks the glamour of a more serious diagnosis such as atypical hyperplasia, and despite its somewhat dull nature, the interpretation can be quite challenging. Because time is limited, not all non-neoplastic endometrial lesions can be covered in this lecture. The emphasis will be on the pathology of **dysfunctional uterine bleeding** (DUB).
Nowadays, the endometrial biopsy for dating in the work-up of infertility, is in decline. Most endometrial biopsies are taken during the investigation of abnormal uterine bleeding (AUB). In peri- and postmenopausal women, the most important indication is to exclude hyperplasia or neoplasia.
The most frequent causes of AUB are DUB, polyps and atrophy. Atrophy is the most common cause of postmenopausal bleeding. DUB is abnormal uterine bleeding in a premenopausal woman, resulting from alterations in the normal cyclic changes of the endometrium, and without an underlying specific organic cause, such as endometritis, polyps, hyperplasia, carcinoma, effects of exogenous hormones, or complications of pregnancy. Clinically, DUB is synonymous with ovarian ovulatory dysfunction.
Basically, the two morphological patterns of the endometrium that are characteristic of DUB, are an abnormal proliferative phase or an abnormal secretory phase. These are most commonly associated with anovulatory or oligo-ovulatory cycles, or less commonly a luteal phase defect (LPD). The alterations can be regarded as estrogen-related or progesterone-related, respectively.
A characteristic feature of biopsies from patients with DUB is the presence of non-menstrual glandular and stromal breakdown. Apoptosis with nuclear debris at the base of glands and within the stroma is an early feature. The stromal cells collapse and aggregate into thight clusters, which are separated by lakes of blood. Fibrin thrombi form in small blood vessels. The clusters of stromal cells, also called “blue balls” or “exodus balls”, may form small polypoid extrusions or become detached from the underlying tissue. The thightly packed cells have hyperchromatic nuclei and scanty cytoplasm. They often retain a cap of epithelial cells. Eosinophilic syncytial change or “papillary syncytial metaplasia”, is an almost consistent feature of breakdown. It is a regenerative process, characterized by syncytial sheets of epithelial cells with indistinct cell borders that form micropapillary structures. There may be mild atypia, and mitoses can be identified. Occasionally, especially in small biopsy specimens, it may be difficult to distinguish between papillary syncytial metaplasia and uterine serous carcinoma. Immunohistochemistry should be used with caution.
Anovulatory cycles are one of the most common causes of DUB. As stated earlier, DUB caused by anovulation is estrogen-related. A low estrogen state results in deficient proliferation. It is caused by sporadic anovulation, which often results in precipitous

atresia of the follicle(s) with estrogen withdrawal bleeding. There is minimal endometrial proliferation. A small amount of endometrium with weakly proliferative glands and stroma develops. Deficient proliferation can also be caused by iatrogenic suppression of ovulation, but by definition this is not DUB.
An unopposed estrogen state results in a disordered proliferative endometrium. It is caused by repeated anovulation, which results in thickening of the endometrium, outgrowth of its blood supply, formation of thrombi in superficial vessels, and estrogen breakthrough bleeding. Repeated anovulation, caused by a persistent unruptured follicle, is a very common cause of DUB in perimenarchal or perimenopausal women. It may also be a component of the polycystic ovarian syndrome. Disordered proliferation shows proliferative glands with focal branching and dilatation. This intermingling of normal and hyperplastic glands represents in fact the earliest stage of simple hyperplasia. Disordered proliferation due to iatrogenic stimulation by exogenous estrogens is by definition not DUB.
DUB can also be caused by a luteal phase defect, meaning a shortened or prolonged luteal phase. The most frequent secretory phase abnormality (or progesterone-related bleeding) is deficient secretion. Sporadic deficient secretion is relatively common in normal women. It must be found in at least two consecutive cycles to be clinically significant. Deficient secretion is the failure of the endometrium to be in the right phase at the right time; the endometrium is "out of phase". Ovulation occurs, but the corpus luteum is functionally inadequate, regressing too early or producing inadequate progesterone (or the endometrium fails to respond), causing either DUB or infertility. Myomas, polyps, adhesions and chronic endometritis may be responsible for suboptimal endometrial response to adequate progesterone, and thus poor secretory changes.
A deficient secretory endometrium can show a plethora of different morphological patterns. The delayed synchronous pattern can only be recognized if the date of ovulation is known (based on basal body temperature, serum progesterone level, or preovulatory follicle size). The requirement for a 3-day delay for the diagnosis of out of phase endometrium highly increases the specificity of the diagnosis. Using a 2-day delay definition for LPD, as originally suggested by G. Jones, is at the limit of interobserver variability for endometrial dating. The glandular stromal asynchrony pattern shows weak, irregular, or just delayed glandular maturation. Stromal maturation is in advance. Deficient secretion with poor secretory changes in the whole endometrium is characterized by undistended, slightly tortuous glands with weak secretory activity, lined by low epithelial cells with dense, oval and palissaded nuclei.
Irregular shedding is a rare luteal phase abnormality, caused by a persistent corpus luteum with prolonged progesterone secretion. It is histologically characterized by a mixed pattern, composed of secretory and proliferative endometrium.
When diagnosing the pathology of AUB, the first step in a practical algorithm is to exclude cancer or precancer. The second step is to classify the cause of the abnormal bleeding, if possible. Is there evidence of breakdown? Reporting breakdown localizes the source of bleeding to the endometrium. Is there an underlying organic lesion such as polyps, endometritis, hyperplasia, pregnancy complication, etc? If yes, diagnose as

such. If no, phase the endometrium (proliferative versus secretory). Then look for subtleties that make the proliferative or secretory endometrium abnormal.
In reporting the DUB biopsy, minimize comments about metaplasia, avoid clinical terms (such as LPD), and use descriptive interpretations, e.g. "Secretory endometrium with premature breakdown", "Weakly proliferative endometrium with breakdown", "Secretory endometrium with irregular maturation". A descriptive diagnosis is the best approach for the pathologist, and provides the most useful information to the gynecologist.

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L 33
THE ENDOMETRIAL INTRAEPITHELIAL NEOPLASIA /EIN CONCEPT.

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Until recently, according to the WHO 2003 classification, both generalized endometrial hormonal changes and localized premalignant lesions were pooled under the term « endometrial hyperplasia » with variants including simple or complex (adenomatous) with or without atypia. This classification is always widely used but molecular studies have provided evidence that the use of the term hyperplasia is conceptually correct for some but not all of the lesions (1). Therefore, the concept of endometrial intraepithelial neoplasia (EIN) which is a clonal proliferation of architecturally and cytologically altered premalignant endometrial cell leading to malignant transformation to endometrioid (type 1) endometrial carcinoma has been proposed (2).
Identically to the others gynaecological classifications (eg CIN,...), if EIN represent the pathological features of premalignant disease of the endometrium, classically therefore these lesions are not divided into subgroups.
The EIN pathological diagnostic criteria modified from Silverberg et al (1,2) are described in the following table.

EIN Criterion	Comments
Architecture	Area of Glands greater than Stroma
Cytology	Cytology differs between architecturally crowded focus and background, or clearly abnormal.
Size >1 mm	Maximum linear dimension exceeds 1mm.
Exclude mimics	Benign conditions with overlapping criteria: Basalis, secretory, polyps, repair, etc..
Exclude Cancer	Carcinoma if mazelike glands, solid areas, polygonal "mosaic-like" glands, myoinvasion, or significant cribriforming

The main differential diagnosis of EIN are respectively reactive changes, artifactual gland displacement, persistent estrogen effect, mid to late secretory endometrium, endometrial polyps, endometrial breakdown and carcinoma.
The classical immunohistochemical biomarker of EIN is the PTEN protein which is lost in about 50 to 60 % of EIN (not in all the cases !) by genomic mutational and/or deletional mutation. However, the use of immunohistochemistry to performe a EIN remains difficult. Firstly, because a lot a commercial antibodies against PTEN do not (or imperfectly) work on paraffin-embedded tissue. Secondly, a loss of of expression is not equated with a loss function. Indeed, by exemple, secretory or atrophic glands show a loss or a reduction of PTEN expression (3). Less frequently (<20%) a lost of MLH1 protein has been described in EIN.
The management of EIN, particularly due to a high concurrent rate of invasive carcinoma (20-30%) is immediate hysterectomy. Therefore, in more young women progestin hormonal therapy can be successful.

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L 34
Histological typing of endometrial carcinoma.

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The histological type is one of the core data items required in the histopathological report of an endometrial carcinoma [1].
Certain features of endometrial carcinoma including the histological type, will determine the type of surgery performed, whether adjuvant therapy will be administered and the choice of adjuvant therapy. Accurate typing is necessary in both biopsies and resection specimens. Diagnosis of aggressive tumours such as serous carcinoma, clear cell carcinoma, carcinosarcoma, undifferentiated carcinoma and grade 3 endometrioid carcinoma will usually result in full surgical staging including pelvic and para-aortic lymphadenectomy and omentectomy at a Cancer Centre.
Core data items including the histological type can be used in conjunction with clinical data to determine prognosis.
Accurate typing of endometrial cancers will also allow epidemiological information to be collected with regard to cancer subtypes and association with genetic syndromes.

Endometrial carcinomas should be typed according to the **2014 WHO classification[2]:**

Endometrial carcinomas
- Endometrioid carcinoma
  - Variant with squamous differentiation
  - Villoglandular variant
- Secretory variant
- Mucinous carcinoma
- Serous endometrial intraepithelial carcinoma
- Serous carcinoma
- Clear cell carcinoma
- Neuroendocrine tumours
  - Low-grade neuroendocrine tumour
    - Carcinoid tumour
  - High-grade neuroendocrine carcinoma
    - Small cell neuroendocrine carcinoma
    - Large cell neuroendocrine carcinoma
- Mixed cell adenocarcinoma
- Undifferentiated carcinoma
- Dedifferentiated carcinoma

Squamous cell carcinoma and transitional cell carcinoma are extremely uncommon histological types; they are no longer mentioned in the 2014 WHO classification.

Carcinosarcoma or malignant mixed Müllerian tumour belongs to the group of mixed epithelial and mesenchymal tumours and will not be discussed here.

Endometrioid carcinoma of the usual type is a glandular neoplasm displaying an acinar, papillary or partly solid configuration, but lacking the nuclear features of endometrial serous carcinoma. The lining cells are usually columnar and share a common apical border with adjacent cells, resulting in a smoothly contoured glandular lumen. Nuclear atypia is usually mild to moderate with inconspicuous nucleoli except in poorly differentiated carcinomas. The cytoplasm is eosinophilic and mitotic index is highly variable.

Between 10 and 25% of endometrioid carcinomas contain foci of squamous differentiation, the recognition of which is important since it is not included in the estimation of solid growth for grading endometrioid carcinoma. The term 'endometrioid adenocarcinoma with squamous differentiation' refers to the entities previously termed 'adenoacanthoma' and 'adenosquamous carcinoma'. It has been shown that the cytomorphology of the squamous elements in such tumours is not a useful prognostic indicator.

Less than 2% of architecturally typical endometrioid adenocarcinomas are composed of columnar cells that have single, large, sub- of supranuclear vacuoles of glycogen rather than eosinophilic cytoplasm, resembling endometrial glands of the secretory phase. These carcinomas are almost always well differentiated.
Less frequent patterns of endometrioid carcinomas include villoglandular, sertoliform and microglandular types. There remain inevitable problems with grading. Many pathologists consider tight, small microacini with barely visible lumens as solid growth, which means that a significant amount of this usually leads to the classification as FIGO grade 2 or 3. And what about spindle cell change? Is this considered as squamous or solid non-squamous?
Immunohistochemistry to distinguish between an endometrial and a cervical adenocarcinoma is more often necessary in biopsies than in resection specimens. Generally, endometrioid endometrial carcinomas are strongly and diffusely positive for vimentin, ER and PR and largely negative for CEA, except in areas of squamous and mucinous differentiation. The converse profile is usual in cervical adenocarcinomas. CEA expression in cervical adenocarcinomas of the usual type is characteristically diffuse with cytoplasmic and luminal border immunoreactivity, whereas endometrioid adenocarcinomas of the uterus may exhibit weak, luminal CEA. p16 staining may also be useful in the distinction between an endometrioid adenocarcinoma of the uterine corpus and a cervical adenocarcinoma: the former is usually patchily positive and the latter diffusely immunoreactive.
More than 50% of endometrioid adenocarcinomas show mutation or inactivation of *PTEN*, resulting in loss of *PTEN* immunoreactivity. About 35% of tumours display microsatellite instability. In sporadic cases this is most often due to hypermethylation of

the *MLH1* gene promotor. In the Lynch syndrome (hereditary non-polyposis colorectal cancer, HNPCC) this is due to germline transmission of defective DNA mismatch repair genes (*MLH1, PMS2, MSH2* and *MSH6*). Pathological features of endometrial carcinomas associated with Lynch syndrome are not well studied but lower uterine segment location, undifferentiated areas and abundant tumour infiltrating lymphocytes may be a feature. Loss of expression of mismatch repair proteins (MLH1/PMS2 or MSH2/MSH6) can be immunohistochemically detected and based on these results, additional testing may follow to determine if the patient has a germline mutation diagnostic of Lynch syndrome.
When early-stage, low- grade endometrioid adenocarcinomas involve the uterus and one or both ovaries, they most likely represent synchronous independent primary neoplasms. Adjacent endometrial hyperplasia (in the case of the uterine tumour) and endometriosis or a component of benign or borderline endometrioid adenofibroma (in the case of the ovarian neoplasm) are pointers towards an origin in these organs. With a deeply myoinvasive endometrial tumour exhibiting prominent lymphovascular invasion and nodular cortical and surface ovarian tumour, a uterine primary with ovarian metastasis is likely.

Mucinous carcinoma is defined as an endometrial carcinoma in which > 50% of the neoplasm is composed of mucinous cells. Many endometrioid adenocarcinomas contain focal mucinous areas and endometrioid and mucinous adenocarcinomas form part of a spectrum. Mucinous carcinoma accounts for only 1-9% of endometrial carcinomas. The architecture is glandular or villoglandular and the lining cells are uniform with minimal stratification. The mucin is recognized as basophilic globules or pale cytoplasm that is positive for PAS after diastase, mucicarmine and CEA. Nuclear atypia is mild to moderate and mitotic activity is low.
Immunohistochemistry may be helpful in the distinction from endocervical adenocarcinoma as described above.
Information about mucinous carcinomas is still relatively limited, but available data suggest that their clinical behaviour is similar to that of endometrioid adenocarcinoma. Since most mucinous carcinomas are well differentiated, prognosis is relatively good.

Serous carcinoma is characterized by complex papillary and/or glandular architecture with diffuse marked nuclear pleomorphism; a solid growth pattern may also occur. A glandular pattern also. When a serous carcinoma invades the myometrium, it frequently displays gaping glands. The neoplastic cells have large atypical nuclei, prominent nucleoli and scant cytoplasm. The luminal surface appears scalloped or frayed since a common apical border is often lacking. Mitotic figures are numerous. Patients are typically postmenopausal with a mean age in the late sixties.
Uterine serous carcinoma has a marked propensity for extra-uterine spread, which may occur even with a small primary tumour apparently confined to the endometrium. In such cases, it is important to distinguish between a primary uterine serous carcinoma with metastasis to the adnexa, a primary adnexal serous carcinoma with spread to the endometrium and independent primaries. Currently it is considered that with a serous

carcinoma in more than one location, these are much more likely to represent metastasis from one site to the other rather than synchronous independent neoplasms. Most ovarian and tubal serous carcinomas exhibit diffuse strong nuclear positivity for WT1; in contrast, uterine serous carcinoma is usually negative, although some cases are positive.
Grade 3 endometrioid adenocarcinomas show clinical behaviour similar to serous carcinomas. Grade 3 endometrioid carcinomas generally show a greater incidence of expression of ER and PR, whilst expression of p53 and p16 is commoner is serous carcinomas. However, there is considerable immunophenotypic overlap and markers may not be of value in an individual case. Serous carcinomas almost always exhibit aberrant p53 staining: intense and diffuse nuclear staining of > 75% of the tumour cells or totally negative staining correlates with TP53 mutation and supports the diagnosis of serous carcinoma. In contrast, p53 staining of variable intensity in < 75% of the tumour cell nuclei correlates with wild type TP53 and therefore the tumour is more likely a high grade endometrioid carcinoma.

Serous endometrial intraepithelial carcinoma (SEIC) replaces the surface epithelium and/or glands of the endometrium without invading the stroma. It develops in atrophic endometrium or in an endometrial polyp. Even in the absence of demonstrable invasion, the carcinoma can shed cells and metastasize widely to extra-uterine sites.

Clear cell carcinoma is a neoplasm composed of polygonal or hobnail-shaped cells with clear or eosinophilic cytoplasm arranged in papillary, tubulocystic or solid patterns with at least focal high-grade nuclear atypia. The papillae are typically short with hyalinized stroma and one layer of neoplastic cells. Mitotic figures are usually numerous. About two-thirds of clear cell carcinomas contain densely eosinophilic extracellular globules or hyaline bodies.
It is an uncommon type accounting for 2% of endometrial carcinomas.
Immunohistochemistry may be helpful in distinguishing clear cell carcinomas from endometrioid adenocarcinomas with clear cells. Both carcinoma types show similar expression of CK7, EMA, CA125, Ber-EP4, B72.3 and CEA. ER and PR expression is usually absent in clear cell carcinoma, while HNF-1β is expressed in most cases. Serous carcinoma of the endometrium can be difficult to distinguish from clear cell carcinoma. Aberrant p53 expression (diffuse and strong or totally absent) and diffuse p16 expression favours the diagnosis of serous carcinoma (overexpression of p16/p53 only occasionally occurs in CCC. ER/PR are frequently negative).
Overall survival data vary greatly in the literature, probably reflecting misclassification of clear cell carcinoma and histological mimics. Most studies report a 5-year survival of less than 50% regardless of stage.

Neuroendocrine tumours are rare, representing < 1% of endometrial cancers. It is a new category in the 2014 WHO classification of endometrial carcinomas defined as a diverse group of neoplasms that share a morphological neuroendocrine phenotype. Only 2 examples of primary low-grade neuroendocrine tumours, carcinoid tumours or well

differentiated neuroendocrine tumour grade 1 have been reported. High-grade neuroendocrine carcinomas of the endometrium comprise both small-cell and large-cell neuroendocrine carcinomas; their morphology is similar to that of their counterparts arising in other organs. The diagnosis is based on morphology and the expression of neuroendocrine markers in at least 10% of the neoplastic cells: chromogranin, synaptophysin, CD56 and cytokeratin in a dot-like pattern. Both small-cell and large-cell neuroendocrine carcinomas can arise within the uterine corpus, with or without an endometrioid component.

Mixed cell adenocarcinoma is defined as an endometrial carcinoma composed of 2 or more different histological types of endometrial carcinoma, at least one of which is of the type II category i.e. serous or clear cell carcinoma. The histological cell types must be recognizable on the H&E stained sections although immunohistochemistry may clarify the presence of the distinct cell types. In the 2014 WHO classification, the minimum percentage of the second component has been set at 5%. It is recommended that all morphological types are mentioned in the pathology report along with the approximate percentage of each component. The most commonly encountered mixture is endometrioid and serous carcinoma. The behaviour of these tumours correlates with the highest grade component. It has been shown that even a minor component of an aggressive tumour type comprising as little as 5% of the neoplasm is of clinical importance.

Undifferentiated carcinoma of the endometrium is defined as a malignant epithelial neoplasm with no differentiation. It is a tumour composed of small to intermediate-sized dyshesive cells arranged in sheets without any obvious nested or trabecular architecture; there is complete absence of squamous or glandular differentiation and only minor foci of neuroendocrine marker positivity (< 10%) are allowable. Most cases have > 25 mitotic figures per 10 HPF. Tumour infiltrating lymphocytes are often numerous. Undifferentiated carcinoma is a specific histologic entity and does not imply that the pathologist cannot supply a definitive diagnosis.
Immunohistochemically, evidence of epithelial differentiation is present in only occasional tumour cells with intense EMA and CK18 expression in the absence of pan-CK immunoreactivity. Tumour cells express vimentin but are negative for ER, PR or E-cadherin. Chromogranin and/or synaptophysin can be present in a minority of tumour cells.

Dedifferentiated carcinoma is composed of undifferentiated carcinoma and a second component of either FIGO grade 1 or 2 endometrioid carcinoma. The differentiated component usually lines the endometrial cavity, while the undifferentiated component grows beneath it.

The **interobserver reproducibility** of histological subtype diagnosis is not perfect even between expert gynaecological pathologists, especially in cases of high-grade endometrial carcinoma.[3]

Good preservation of tumour morphology is of crucial importance for accurate subtyping and grading of any tumour. Endometrial carcinomas are particularly susceptible to autolysis. The specimen should be transported to the laboratory as soon after surgery as possible. Whether received fresh or in formalin, the uterus should be opened as soon after receipt as possible in order to facilitate fixation of the tumour and preservation of tumour morphology.

Ancillary investigations, especially immunohistochemistry but also increasingly molecular tests, aid in the accurate and reproducible diagnosis of an endometrial carcinoma subtype. A combination of antibodies is usually required to make a diagnosis: ER, PR, p53, p16, Ki-67, HNF-1β, panCK, PTEN, CEA and vimentin. They should be used in concert with thorough morphologic examination, as part of rational panel of markers and only in specific circumstances.[4]

Hormone receptor (ER and PR) status may also have predictive value in the management of recalcitrant or recurrent disease or in the management of low-grade adenocarcinomas where surgery is contraindicated, for example due to comorbidities or desirability to preserve fertility.

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L 35
Grading, staging and molecular classification of endometrial carcinoma.

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Histopathologic correct grading and staging of endometrial malignancies is of utmost importancy for correct treatment of the patient. Postoperatively, patients are classified into low, intermediate or high risk based in this surgical pathologic staging. Patients with grade 1 or 2 tumors, confined to the endometrium or only minimally invasive, are defined as low risk and do not require further therapy. Almost solely based on the report of the pathologist, the gynecologic-oncologist will decide if a patient will be treated with surgery alone, or if she will receive adjuvant radiation- and/or chemotherapy.

Histologic grade is highly correlated with other prognostic factors, such as age, stage and depth of myometrial invasion. However, in multivariate analysis, grading has been found of important value as even for patients with metastatic disease (stage III), the histologic grade was significant in predicting outcome, as an independent variable. By definition, serous carcinoma, clear cell carcinoma and carcinosarcoma of the endometrium are defined as grade 3. Villoglandular and mucinous endometrial carcinomas are (usually) grade 1 tumors. Endometrioid endometrial carcinoma is divided in a 3-tier system based on solid growth: less or equal to 5% defines grade 1, between 5 and 50% is grade 2 and above 50% is a grade 3 tumor.

Staging for endometrial carcinomas (and carcinosarcomas) was revised in the 2009 FIGO staging system (see table). Major changes from the staging system of 1988 include the inclusion of tumors with no myometrial invasion and those with inner half myometrial invasion in stage IA, elimination of cervical gland involvement from stage II, elimination of positive peritoneal fluid cytology from stage IIIA, and separation of pelvic from para-aortic lymph node metastases within stage IIIC. Correct examination of myometrial and cervical involvement may sometimes be difficult to distinghuish, and it may require taking additional slides. Patterns of myometrial invasion A major risk factor, however not included in the staging system, is (extensive) lymphovascular space invasion. At present, there is debate regarding the necessity for complete surgical staging of all women, as lymphadenectomy is only performed in patients with high risk for nodal metastases.

In the past, endometrial cancers have traditionally been subdivided in type I and type II on the basis of differences in histology and clinical outcomes. Type I tumors comprise the large majority of endometrial cancers, are mostly endometrioid adenocarcinomas and are associated with unopposed estrogen stimulation, obesity and endometrial

hyperplasia. Type II tumors are predominantly serous carcinomas, arising in atrophic endometrium. The disparate genetic alterations found in type I and type II tumors suggested that these subtypes have distinct etiologies.

Stage I		**Tumor confined to corpus uteri**
	IA	No or ½ myometrial invasion
	IB	≥ ½ myometrial invasion
Stage II		**Tumor invades stromal connnective tissue of the cervix but does not extend beyond uterus**
Stage III		**Local and/or regional spread of tumor**
	IIIA	Invades serosa and/or adnexa
	IIIB	Vaginal and/or parametrial involvement
	IIIC	Metastases to pelvis and/or para-aortic lymph nodes
	IIIC 1	*Positive pelvic lymph nodes*
	IIIC 2	*Positive para-aortic lymph nodes*
Stage IV		**Tumor invades bladder and/or bowel nucosa and/or distant metastases**
	IVA	Tumor invasion of bladder and/or bowel mucosa
	IVB	Distant metastases, including intra-abdominal metastases and/or inguinal lymph nodes

However, it has become increasingly clear that endometrial cancer comprises a biologically, clinically, morphologically and genetically heterogeneous groupe of tumors. The Cancer Genome Atlas Research Network recently reported a comprehensive Integrated Genomic Characterization of Endometrial Carcinoma. They recognized four categories of endometrial carcinoma: POLE ultramutated, microsatellite instability hypermutated, copy number low, and copy number high. Reclassification of endometrial cancer with this system, might directly affect treatment decisions and guide clinical trials. In this era of molecular pathology, it is more and more important for the practicing pathologist to recognize specific (and less specific) histologic features associated with Lynch syndrome and other hereditary syndromes.

Next-generation sequencing and epigenetic profiling are some of the future challenges that will expand our knowledge of signaling pathways of endometrial cancer. Understanding the clinical relevance of specific mutations of rare variants, combined with the classic anatomical and histologic parameters of endometrial carcinoma, will hopefully eventually lead to individualized targeted therapies for these patients.

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L 37
Squamous lesions and their look-alikes

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Cervical cancer represents the third most common cancer among women and a leading cause of mortality worldwide, with 265.653 deaths estimated in 2012[1]. Eighty-three percent of cases occur in the developing world, where cervical cancer accounts for 15% of female cancers, as compared to just 3.6% in developed countries[2].

The large decline in cervical cancer incidence and mortality in high-income countries is largely credited to effective screening programs[3] and Pap test, which is usually reported following Bethesda System 2001. In this classification, we find three general categories: negative for intraepithelial lesion or malignancy, epithelial cell abnormality, and "other". Epithelial cell abnormalities can be glandular and squamous, which include squamous intraepithelial lesions (SIL), squamous carcinoma and atypical squamous cells (ASC). Squamous intraepithelial lesions are divided into low-grade (LG) and high-grade (HG) on the base of the origin of the cells (superficial, intermediate or basal/parabasal) and the degree of nuclear abnormalities.

Low-grade squamous intraepithelial lesions include the presence of mildly dysplastic superficial or intermediate cells and /or cells showing the cytopathic effects of HPV infection, known as koilocytes. These cells show enlarged, usually hyperchromatic nuclei with slightly irregular chromatin pattern resulting in a raised nucleo-cytopasmic ratio (nuclei are two or three times the size of that of a normal cell); the nuclear membrane may be unevenly thickened, notched or wrinkled and nuclei are often asymmetrical and dysmorphic. Koilocytes occur in a great number of cases (but not always) confirming the close relationship of the lesion to HPV infection. They are characterised by the presence of a sharply demarcated perinuclear clear zone, or halo, surrounding single or double nuclei that are usually pyknotic and may show the same features as above. The presence of koilocytes is sufficient to have a SIL, but there are some other conditions in which halo-cells can be misleading. These include the so-called "pseudo-koilocytosis", which we can found in glycogen storage in intermediate cells, radiation effects and some infections, like Trichomoniasis. It is important to identify the nuclear abnormalities of LG-SIL, which are not present in other conditions. In addition, the perinuclear halo of koilocytes is large and well demarcated, hard-edged, unlike that found in other conditions. Multinucleation can be found in HPV infected cells, but it may be present also in herpes virus infection, recognizable by the ground-glass aspect of nuclei, and reactive cellular changes associated with radiotherapy, in which nuclei are enlarged, but show evenly distributed chromatin.

High-grade squamous intraepithelial lesions are characterised by a population of moderately sized parabasal (CIN 2) or basal (CIN 3) dysplastic cells with marked nuclear abnormalities occurring singly or in clusters. These cells typically show large hyperchromatic nuclei with abnormal chromatin pattern and irregular contours, but are seldom hypochromatic (pale dyskariosis).

Despite of all these aspects, identification and correct interpretation of diagnostic cells could be challenging and there are some differential diagnoses that must be taken into consideration.

The finding of parabasal or basal cells in a Pap smear is not sufficient for a diagnosis of H-SIL, because these cells may derive from the epithelium of the squamo-columnar junction or be the consequence of squamous metaplasia. Basal dysplastic cells can be mistaken for immature lymphocytes in a follicular cervicitis due to the similar size and the scant cytoplasm, or macrophages, because of the similar size and arrangements on strings or files. Generally, the key for the correct diagnosis is an accurate examination of nuclei and nucleo-cytoplasmic ratio, which is typically increased in dysplastic cells; the presence of abnormal mitoses can be helpful too.

Repair cells can also be source of confusion, but they are characterised by active nuclei with nucleoli, which are usually absent in dysplastic lesions; moreover, chromatin is evenly distributed in repair cells.

High-grade squamous dysplastic lesions involving glandular structures may exfoliate in three-dimensional aggregates arising diagnostic doubts with glandular lesions. In these cases, the key morphologic features for differential diagnosis could be a close examination of the cytoplasm, which is dense in squamous cells, while is often vacuolated in glandular cells. Furthermore, the disposition of nuclei (which are central in squamous cells and eccentric in glandular cells), the absence of typical architectural features of glandular lesions (feathering, pseudostratification) or the presence of well-defined squamous dysplasia elsewhere on the slide, can represent other useful tools for the correct diagnosis.

Large layers of hyperchromatic cells are a common difficulty for cytologists since the crowding and overlapping elements can render difficult the correct examination of nuclei. In some cases individual dysplastic cells may be present away from the layers and make the diagnosis easier; otherwise, high power examination of cells in the periphery of the clusters, where chromatin and nucleo-cytoplasmic ratio is interpretable, should be undertaken. Architectural features of the cellular layers (disorganisation, loss of nuclear polarity, overlapping, presence of mitoses) may also be helpful.

High-grade keratinising squamous lesions are characterized by keratin-forming dysplastic cells of different shapes with abundant orange or yellow thick cytoplasm. "Tadpole" (caudate) cells, spindly shaped cells and squamous "pearls" can be also observed. Nuclei are often pyknotic or sometimes they can be completely or partially

replaced by keratin ("ghost" cells). Excessive anucleated keratin material coming from the superficial part of lesions could hide either high-grade lesions or benign leukoplakia. In these cases, an adequate colposcopic evaluation, eventually with biopsies, becomes important. Keratinizing high-grade lesions could also be hard to differentiate from invasive cervical cancer; the detection of a necrotic background in invasive lesions is helpful for diagnosis. On the other side, clean background is more suggestive for intraepithelial lesions (in conventional smears).

In particular conditions such as postmenopausal atrophy, cervical epithelium can be severely altered by mucosal dryness. The lack of estrogenic stimulation prevents the complete maturation of the epithelium, so that intermediate and parabasal cells are often present on the smear. Furthermore, squamous cells may appear enlarged or elongated (as it may happen in case of bad spreading on smears), isolated or disposed in hyperchromatic crowded groups, suggesting the presence of a dysplastic lesion even to experienced observers. If nuclear sizes and texture of suspect cells are closely similar to those of benign cells, high-grade SIL diagnosis is not suggested. The presence of mitoses should never be ignored, for their presence is not associated with atrophy and is more suggestive for a dysplastic lesion. If doubts persist, the simplest solution will be to refer patient for colposcopy with ASC diagnosis.

Invasive squamous carcinoma can be suspected in conventional Pap smears in presence of highly atypical cells in a background of necrotic material, blood and debris ("cancer diathesis"), which are the result of cancer infiltration of the underlying stroma. On the other hand, this material could render the diagnosis difficult, obscuring the often poorly preserved diagnostic cells.

Not all invasive cancers show cancer diathesis, particularly those in early stages without surface necrosis. In such instances, the cell sample may be difficult to interpret as invasive tumour.

Invasive cancer cells are often characterised by pleomorphic bizarre forms, markedly hyperchromatic nuclei with extreme abnormalities of chromatin pattern and macronucleoli. Cannibalism aspects are common, giving the appearance of "cell-in-cell".

Caution should be used in presence of only prominent nucleoli as these may be seen in normal reparative changes or in radiotherapy alterations. While macronucleoli are consistent with repair reaction, these entities tend to remain in groups and sheets whilst the discohesiveness of cancer means that in these cases many single cells can be seen. Dysplastic cells, usually derived from areas of intraepithelial lesions on the margin of invasive cancer, may be present in cytological preparations from invasive squamous carcinomas and are seldom the only evidence of disease. However, the predominant abnormal cells are differentiated and undifferentiated cancer cells, displaying marked aberrations of the nucleus and the cytoplasm. This heterogeneous population of cancer cells is very characteristic of invasive cervical cancer.

The cytological identification of the three principal patterns of invasive carcinoma in smears is sometimes possible but is of limited clinical value. However, certain basic differences in cell types may be observed. Keratinizing (squamous) carcinomas shed mainly differentiated, keratinized, squamous cancer cells, some of which may closely resemble dysplastic cells. Koilocytes may be present. In such tumours, squamous "pearls", spindly cancer cells, tadpole cells and other bizarre cell types are common. Small cell carcinomas usually shed a relatively monotonous population of undifferentiated, small malignant cells, occurring singly or in small clusters. Small columnar-shaped cancer cells with cytoplasmic vacuoles are possible and, when numerous, they can mimic those of an adenocarcinoma. In the endocrine variant, arrangement of cancer cells in rosettes may be occasionally observed, but this is a very rare finding.

Atypical squamous cell category includes all the situations in which a reliable interpretation of SIL cannot be made although the cells contain features that are more marked than merely reactive changes. In the revised version of Bethesda System 2001, ASC is divided into two subcategories: atypical squamous cells "of undetermined significance" (ASC-US) and atypical squamous cells, "cannot exclude high-grade SIL," (ASC-H). ASC-US is the more numerically prominent qualifier and accounts for 90 to 95 percent of all ASC results. The use of the terms "undetermined significance" emphasizes that a specific diagnosis cannot be made and that further triage may be appropriate, excluding cytology suggestive for high-grade SIL. On the contrary, ASC-H category includes cytologic changes that are suggestive of high-grade SIL but lack criteria for definitive sure interpretation[4]. The positive predictive value for CIN 2/3 in ASC-H is higher than in ASC-US, but not as high as in the the category H-SIL[5].

Management of ASC varies in different realities. While there is a general agreement on the necessity of colposcopy after an ASC-H diagnosis, ASC-US may require the execution of HPV test or a repetition of the Pap test. HPV test for high-risk subtypes has been proven to have high sensitivity for detection of CIN 2/3 and cancer in combination with Pap test, and it represents an alternative to colposcopy[6]. Women who test positive for HPV should undergo colposcopy[7]. An alternative for women with ASC-US diagnosis is that of repeating the Pap test in one year. If this test is normal, the woman can return to regular screening, otherwise a colposcopy should be done.

Recently, some new studies are investigating the utility of other means in the management of ASC cases. Basing on the fact that high-risk HPV infection indirectly induces the overexpression of p16 protein and an abnormal proliferation in squamous cells[8], dual immunocytochemistry stains for p16/Ki-67 should be used in the daily practise to identify potentially evolving lesions. These methods show comparable sensitivity but significantly higher specificity compared to testing for high-risk HPVs and for p16 alone[9].

Even if Pap test is not perfect and false-negative results cannot be eliminated because of various factors, which limit test sensitivity, we would like to stress the importance of

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

In Trieste, in the last 28 years, invasive squamous cervical carcinoma incidence has had a constant decrease, falling from more than 20 cases/year in 1984 to 5 cases/year in 2012, thanks mainly to the progressive spreading of Pap test.

Good results obtained by cervical screening in our area are also demonstrated by one of our studies starting from 124 invasive cervical cancers observed in Trieste area from 1999 to 2012. Most (70%) of the patients had never been given a Pap smear, 15% had not followed Italian Cervical Screening guidelines, and 3% had been diagnosed a HG lesion through cytology, but they did not respond to second level exams (colposcopy, biopsy and therapy).

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

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L 38
Glandular lesions and their look-alikes.

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Despite the significant reduction in incidence and mortality of squamous cell cervical carcinoma worldwide (and especially in most developed regions), we are observing a steady situation or even a slight increase for endocervical adenocarcinoma.

Endocervical glandular abnormalities, which represent an increasing number of abnormal Pap smears, partly because of improved sampling of the endocervix with the use of cytobrush, are usually reported using the Bethesda System classification, which was significantly revised in the 2001 updating reflecting a reappraisal of the strengths and weaknesses of cytology in assessing these findings.

In cytologic preparations, normal endocervical cells may be present in clusters which aspect depends on the groups' orientation on the slide: they may show "honeycomb" disposition when seen on-end, or as elongated columnar "palisade" cells when seen from the side. Nuclei of normal endocervical cells are round to oval, with evenly dispersed and finely granular chromatin and small to medium-sized nucleoli.

Glandular cells abnormalities are now classified into different categories to help direct the correct clinical management.

Endocervical Adenocarcinoma In Situ (AIS) has been introduced by Bethesda System 2001 as an independent diagnostic category and all the cytomorphological criteria for diagnosis have been outlined. Characteristic cellular arrangement of atypical endocervical elements called "feathering of cells" and their multilayering and overlapping, absence of prominent nucleoli in cells with altered nucleo-cytoplasmic ratio, with chromatin slightly altered but not coarsely distributed, and absence of tumoral diathesis (in conventional Pap smears) represent the key to make AIS diagnosis1. Cells maintain the columnar appearance with respect of polarity and palisading aspects and mitotic figures are also present.

Adenocarcinoma is characterized by the presence of neoplastic elements showing nuclear changes comprising enlargement, hyperchromasia, coarse granulation of chromatin with clearing areas, and large, irregular, sometimes multiple nucleoli. Dyscohesion is more evident than with AIS and isolated tumour cells are commonly seen. Cancer cells often form spherical or oval clusters and the smear background often shows blood, necrosis, and cell debris in conventional Pap smears[2].

Bethesda System 2001 asks, if possible, to specify if the Adenocarcinoma is endocervical, endometrial or extrauterine; some cytomorphologic criteria can be useful to distinguish endocervical adenocarcinoma from endometrial lesions. Endocervical adenocarcinoma

has a tendency to shed more cells on Pap smears, cells are large and tend to preserve a columnar aspect, but as the neoplasm becomes less well-differentiated, the features become ill-formed and may be ultimately lost.

On the other side, endometrial adenocarcinoma sheds less cells on Pap smears, and these are smaller and more round shaped, usually disposed in morules, acinar or papillary formations. The cytology is dependent on the grade and type of tumour from which the cells are derived with nuclear morphology varying from relatively uniform in well-differentiated grade 1 tumours to highly pleomorphic from poorly differentiated adenocarcinomas.

Extrauterine adenocarcinomas detected on Pap smears are rare, but more often due to infiltration of adjacent tumours i.e. rectal adenocarcinoma (in this last case we may observe on cytological smear the typical cellular "palisading" arrangement which resembles the histological aspects), or to metastasis from distant tumours (sometimes from ovarian origin, or also from breast tumours).

Atypical Glandular Cells NOS, either endocervical, endometrial, or glandular cells not otherwise specified, include cytological findings that are common in lesions as polyps, hyperplasia of endocervical epithelium, tubal metaplasia, endometriosis, dysfunctional endometrium, reparative changes following cone biopsies and sampling of the upper endocervical canal/lower uterine segment[3]. The diagnosis of atypical glandular cells is made in presence of slight atypias (nuclear enlargement without chromatin abnormality), in columnar cells shed either isolates or in clusters.

Atypical glandular cells, favour neoplasia, includes those cases with atypias that quantitatively or qualitatively fall short of definitive diagnosis of adenocarcinoma. Benign and malignant glandular cytologic features sometimes overlap. Reactive metaplastic cells, atrophy, radiation effects and chemotherapy changes may all mimic glandular lesions. Awareness of these potential pitfalls and recognition of their specific cytologic criteria may help in the identification of the cause of endocervical atypia and minimize its over interpretation.

Reactive cellular changes are associated with inflammation, radiation, repair and other non-specific causes and can occur both in the squamous metaplastic and endocervical cells. In these situations, endocervical cells may show markedly enlarged, hypochromic nuclei with prominent nucleoli, and are commonly multinucleated. Nuclear membrane is thickened, round and smooth; chromatin is fine, granular and evenly distributed.

Tubal metaplasia is the most common benign process that can lead to "atypical" cells mimicking an endocervical neoplastic process. Cells sampled from this process generally have larger, more hyperchromatic nuclei than do normal native endocervical cells. In addition, they may show some of the architectural features of adenocarcinoma in situ, including pseudostratification, rosette formation, and nuclear marginal protrusion

(feathering). Presence of ciliated cells in the groups, or goblet cells filled with mucin are very helpful in assigning a benign origin to a group. In addition, tubal metaplastic epithelial nuclei almost never show the coarsely granular chromatin pattern of AIS.

Endometrial cells may also be sampled from the lower uterine segment by the new sampling devices. These cells generally are smaller and have smaller nuclei than do endocervical cells, and also have a much higher nucleo-cytoplasmic ratio. With direct sampling of the endometrial cavity, large two-dimensional clusters of endometrial cells may also show columnar morphology and pseudostratified architecture, as opposed to the more familiar three-dimensional tightly clustered groups of endometrial cells, which are seen in spontaneous exfoliation. In addition, when proliferative endometrium is sampled, frequent mitotic figures and even apoptotic bodies may be noted, indicative of active cell proliferation. Close attention to nuclear size (much smaller in EM) will permit appropriate categorization.

The separation of AGC into AGC-NOS and AGC, favour neoplasia, was included in the 2001 Bethesda System since these categories appear to be at different risk for having high-grade cervical neoplasia or cancer. Women with an AGC-NOS result have a 9-41% risk of having a high-grade lesion and a 0-15% risk of having either adenocarcinoma in situ or invasive carcinoma[4].

In contrast, women with an AGC, favour neoplasia result have a 27-96% risk of having high-grade lesion and a 10-93% risk of having either adenocarcinoma in situ or invasive carcinoma. A cytological result of AIS is associated with a 48-69% risk of biopsy confirmed AIS and a 38% risk of invasive adenocarcinoma. Because of this differing risk, women with AGC-NOS are managed less aggressively than women with an AGC, favour neoplasia result or a cytological diagnosis of AIS.

Almost all series of women with AGC results have reported, somewhat paradoxically, that the single most frequent histopathological abnormality identified in these women is CIN 2/3, because high-grade squamous lesions involving glands mimick atypical glandular cells. Some cytomorphologic criteria can be helpful to distinguish between squamous lesions involving endocervical glands and dysplastic glandular elements. If we observe three-dimensional aggregates on Pap smear and we notice a tendency of cells to “escape” from the centre of the aggregate, it is more likely that they are endocervical elements, as squamous dysplastic elements involving glands tend to crowd in the centre of the aggregate. Besides that, endocervical elements display nuclei at the cell periphery, with cytoplasmic vacuolization, while squamous elements tend to have more centrally located nuclei and cytoplasms are more often dense. Based on the high prevalence of CIN 2/3 in women with AGC the 2001 Consensus Guidelines recommend that all women with an AGC cytological result have to be referred for a colposcopic evaluation[5]. Moreover, since it can be quite difficult to identify AIS based on colposcopic appearance alone and AIS lesions can be confined to the endocervical canal, the 2001 Consensus Guidelines recommend that the colposcopic evaluation of women with AGC be accompanied by endocervical sampling.

Immunostaining for p16 and Ki-67 may be helpful also for the diagnosis of glandular lesions of the cervix uteri. In particularly problematic cases, the combination of p16 and a proliferation marker can provide additional help for the interpretation of these lesions[6]. We must remember that also ciliated cells in tubal metaplasia are positive for p16 and could be misleading in the use of this marker in daily practice.

Age is another key indicator for determining both the type and frequency of neoplasia identified in women with AGC. Premenopausal patients with AGC are much more likely to have a CIN 2/3 lesion or AIS than to have endometrial disease. In contrast, postmenopausal patients are more likely to have endometrial disease including endometrial adenocarcinoma than CIN2/3 or AIS. Based on the relationship between patients age or menopausal status and risk for endometrial neoplasia, the 2001 Consensus Guidelines recommend that initial evaluation for all non pregnant women with ACG be colposcopy with endocervical sampling and that this be accompanied by sampling of the endometrium in women greater than 35 years of age and in all women with AGC with abnormal uterine bleeding[7].

Of greatest importance in the management of endocervical glandular lesions is the understanding that Pap test is an imperfect tool for detecting these lesions, as its main role is to fight and to prevent invasive cervical squamous carcinoma, even if glandular abnormalities are often associated with squamous lesions. Using of Bethesda System to report our cytological diagnosis promotes more effective communication from the laboratory to the clinicians to provide more uniform, evidence-based, care of women with cervical abnormalities.

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L 42
MINIMAL DATA SET TO OBTAIN OPTIMAL REGISTRATION DATA FROM PATHOLOGISTS.

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Introduction

Whenever a hospital or a laboratory for pathologic anatomy diagnoses or treats a patient with cancer, they are both required by the law of 13/12/2006 (1) to register the case and to transfer this registration to the Belgian Cancer Registry (BCR). Since 2010, the pathologists are obliged to additionally register the diagnoses of the samples taken in the context of early cancer detection (2).

In that regard, all pathology laboratories receive data requests from the BCR at regular times. These requests encompass 4 projects: data related to Cancer and data related to Prevention (Breast, Colon and Cervix). For each project a structured file and a protocol file (text) are required.

Data collection and data transfer is followed by an extensive Quality Control of the received information in a dedicated application. To enable this crucial data cleaning, data delivery should be as adequate as possible. The use of a fixed format and specific classification, as made obligatory by the law of 2006, is therefore of utmost importance.

Accepted classifications for pathology laboratories

For Dutch speaking laboratories the CODAP-2007 classification has been developed, while French speaking laboratories can use the SNOMED 3.5 VF, by courtesy of the French government and this free of charge. As different classifications entail slightly different datasets (for Dutch/French speaking laboratories), the number of used coding languages should be limited to an absolute minimum.

Datasets for the different projects (Cancer/Prevention)

The variables included in the dataset of the structured file are limited to those considered indispensable and suitable to be assessed. The dataset for the **Cancer file** is based on datasets used by cancer registries over the world according to international guidelines. The dataset for the **Prevention files** is based on the needs *of the different centers responsible for organized cancer detection in Belgium*.

While the data of the Cancer files are completely managed by the BCR (from the reception till the publication of the results), the information of the Prevention files is used to support/sustain the centers responsible for organized cancer detection in Belgium in obtaining their goals.

As a manual extraction of the demanded dataset information from the pathology protocols at the BCR would be labor extensive, pathologists are asked to actively collaborate by providing encoded information in separate fields. Accurate encoding is necessary to enable dedicated IT-applications to extract and gather these fields into a coded data file.

Short description of the different variables present in one or more datasets
Within the dataset of the structured file, some variables are compulsory while others are optional. The content and format of the different variables is well specified in a document "BCR-protocol" that accompanies every data demand.

The identification of the patient is of upmost importance. Preferably, the National Social Security Number (INSZ/NISS) is provided, which the BCR is authorized by law to use as the unique patient identifier. In case the INSZ/NISS is lacking, one can deliver the last name, first name, sex, date of birth and postal code.

Date of death may be registered (optional variable) but is mostly unknown by the pathologist.

The country code is an important and obligatory variable because only patients with an official residence in Belgium are eligible to be included in the incidence numbers of Belgium.

The specimen number or protocol number is indispensable to link the structured data to the corresponding accompanying protocol present in the protocol file.
The date the specimen is taken is mandatory, as this date is used to establish the incidence date (date of the first microscopic evidence of the malignant tumour). Without a sample date, it is unclear which incidence year must be taken into account and as a consequence makes the registration untreatable.

The requesting hospital (being the hospital that asked for the pathology examination) is an optional variable. Some important information as laterality, the segment of the colon or the real incidence data is not always available to the pathologist. With the help of this variable, the BCR data managers can easily identify the center from which additional information can be obtained.

The diagnostic procedure is an optional variable that is only present in the dataset for SNOMED users, and refers to the procedure that has led to the microscopic cancer diagnosis.
For Cancer files, this variable enables to distinguish between cytology and histology, and to know if the diagnosis has been made on the examination of the primary tumour or its metastasis. For the Prevention files (cervix), this variable adds in establishing the

amount of diagnoses based on pap smears and in verifying if an aberrant pap-result is followed by a biopsy (fail safe mechanism). The variable also helps to examine which cytological results triggered a biopsy.

The organ code is the localization code of site from which the diagnostic sample was taken. This can be the localization of the primary tumour but also the place of nodal or distant metastasis. It is recommended to provide detailed information about the specific place on the skin, in the colon, …. because, in conformity to specific cancer registration rules, tumours occurring at different, well-defined segments on the skin or colon are considered and registered as multiple tumours.

The lesion code (CODAP) or the **morphology code** (SNOMED), provides information on the cytology/histology of the lesion.

When a primary tumour has been resected, it is sometimes possible to determine a pTNM. Although since several years, the **pTNM** classification is mostly present in the pathological reports, a manual extraction of the information by the BCR data managers is very time-consuming. Additional tools such as textual recognition are sometimes useful but inevitably associated with poorer data quality. Therefore, the collaboration of the pathologist is asked to provide this information in a coded data field.

A pathologist can inform us about the certainty of the diagnosis in an optional field.

Some variables are specific to the cervix Prevention file, such as the quality of the specimen and the variables describing the HPV test results.

The **quality of the specimen** (of pap smears) is important when one wants to set up a fail-safe system in which patients with an abnormal result or with a smear that not allows an evaluation, get the adapted care.

As cervical cancer is **HPV**-related and prevention campaigns include vaccination of young girls, data on HPV test results are very informative. In case an HPV test is performed, the presence of HPV, ideally including details concerning the detected subtypes, should be communicated if possible.

The Cancer Registry uses the **nomenclature numbers of the cervixfile** to make the difference between cytology and histology (extra control), screening and follow up, first exam or second reading,… The nomenclature numbers are also used to create correct exclusion lists for the cervical screening program to prevent unnecessary invitation of patients from which in fact an cervical smear was taken, but not always reimbursed by the health insurances (using only the data of the health insurances would invite too many women unnecessarily).

The nomenclature numbers provided by the pathologists will only be used in the BCR and never be communicated to external partners with a link to a certain lab.
This variable has been recently modified from obligatory to optional because of interpretation problems.

Creation of files for the Belgian Cancer Registry
The creation of the different **structured files**, asked for by the BCR, is depending on the use of specific inclusion criteria.
For the Cancer file, the inclusion criteria are based on the lesion/morphology codes regardless of the sample in which a certain pathology was found.
For the Prevention files, the inclusion criteria are based on the organ codes, regardless of the microscopic findings.

This has important consequences :

- Prevention files also contain negative test results or samples not allowing to make a diagnosis (eg because of insufficient quality of the sample)
- detected cancers of breast, cervix and colon will be present in two files : the Cancer file and the specific Prevention file.
- Absence of an organ code will make extraction for a Prevention file impossible
- Absence of a lesion/morphology code will make extraction for a Cancer file impossible.

Each structured file has to be accompanied by a **separate file** in which the according protocols are gathered. These protocols have to be anonymized and foreseen by a unique protocol number to enable linkage to the coded information of the structured file.

What will be done with this information ?
Cancer file : once data of the laboratories for pathologic anatomy or the oncological care programs are received at the BCR, they are made ready for import in the BCR-specific application. The data belonging to the same patient (INSZ/NISS) is coupled and compared to end up with **one registration for each primary tumour. This registration needs to be as complete as possible.** 75% of all received records are treated in an automated way, but yearly about 120.000 of the 480.000 received cancer related records are investigated more in depth. With the help of these detailed revisions, a Cancer Registry database of high quality is established.
Data from the Cancer Registry database are used to obtain incidence and prevalence numbers per year, region, tumour type, tumour stage, to perform tumour-specific survival analyses, to calculate Quality-Indicators for different tumour types with special interest in the diagnostic and therapeutic procedures, to create maps on (trends in) cancer incidence, to support research,…

Data of the colon file will be used to evaluate the recently started screening program e.g. to get an idea of the consequences of a positive iFOBT or a negative one, to learn more about interval cancers (cancers detected after a negative colonoscopy and before the next planned screening), …

Data of the breast file are compared with the results of the mammography results to study the consequences of positive/negative radiologic features, to calculate quality indicators and to get insight in interval cancers (cancers detected after a negative mammography and before the next screening examination).

Data of the cervix file are used to obtain an efficient *call-recall invitation model* to support the screening program. This includes the creation of exclusion lists, in order to limit the invitations to those women who really need a pap smear (eg all women who got a pap smear in recent history – regardless of reimbursement by the government - are excluded). Data are also used to set up a *fail-safe mechanism* : is an aberrant pap-result followed by adequate diagnostic or therapeutic actions ? Which actions are undertaken in case of an insufficient sample that not allowed any evaluation ?

As forewarned is forearmed, we hope that this additional information about the dataset and the underlying reasoning behind, will enhance the motivation to deliver the most complete and accurate data, including the expected format. As clarified, all of the delivered information is considered valuable, and is used for a huge variety purposes. In order to establish a firm collaboration with a limited help of IT-staff, the minimal data sets will be kept unchanged as long as possible. The accompanying document (the so called BCR-protocol) may be adapted e.g. to include extra explanatory information.

(1) Wet houdende diverse bepalingen betreffende gezondheid van 13 december 2006, artikel 39. Belgisch Staatsblad, 22 december 2006 ; Loi portant dispositions diverses en matière de santé du 13 décembre 2006, article 39. Moniteur Belge, 22 décembre 2006.
(2) Wijziging van de Wet houdende diverse bepalingen inzake gezondheid van 13 december 2006, Belgisch Staatsblad, 2 juni 2010 ; Modifications de la Loi portant dispositions diverses en matière de santé du 23 décembre, Moniteur Belge, 2 juin 2010.

Table 1 : dataset for CODAP-users for the different projects

	VARIABLES FOR CODAP users	DATASET FOR CANCER DIAGNOSES	DATASET FOR BREAST AND COLON PREVENTION FILE	DATASET FOR CERVIX PREVENTION FILE
		following international guidelines for cancer registries	*According to the need of the Centers for Cancer Detection in Belgium*	
1	National Social Security Number (INSZ/NISS)	C	C	C
2	Last name	O/C	O/C	O/C
3	First name	O/C	O/C	O/C
4	Sex	C	C	C
5	Date of birth	C	C	C
6	Date of death	O	O	O
7	Zip code = postal code	C	C	C
8	Country code	C	C	C
9	Specimen number	C	C	C
10	Date specimen was taken	C	C	C
11	Requesting hospital	O	O	O
12	RIZIV/INAMI number of the demander of the test		C	C
13	Quality of the specimen			C
14	Organ	C	C	C
15	Lesion	C	C	C
16	pT	O/C*		
17	pN	O/C*		
18	pM	O/C*		
19	Degree of certainty	O	O	O
20	HPV **high risk** test results			C if HPV test performed
21	HPV **high risk** types detected			O
22	Nomenclature number(s)		O	O

O = optional ; C = compulsory
O/C : compulsory if INSZ not delivered ; optional when INSZ is delivered
O/C* : only compulsory if TNM-classification applicable for the coded specimen
/// : not applicable

For details on the specific variables, see BCR-protocol (www.kankerregister.org or www.registreducancer.org).

Table 2 : dataset for SNOMED-users for the different projects

	VARIABLES FOR SNOMED USERS	DATASET FOR CANCER DIAGNOSES	DATASET FOR BREAST AND COLON PREVENTION FILE	DATASET FOR CERVIX PREVENTION FILE
		following international guidelines for cancer registries	*According to the need of the Centers for Cancer Detection in Belgium*	
1	National Social Security Number (INSZ/NISS)	C	C	C
2	Last name	O/C	O/C	O/C
3	First name	O/C	O/C	O/C
4	Seks	C	C	C
5	Date of birth	C	C	C
6	Date of death	O	O	O
7	Zip code = postal code	C	C	C
8	Country code	C	C	C
9	Specimen number	C	C	C
10	Date specimen was taken	C	C	C
11	Requesting hospital	O	O	O
12	RIZIV/INAMI number of the demander of the test		C	C
13	Quality of the specimen			C
14	Diagnostic procedure	O or HR	O or HR	HR
15	Organ	C	C	C
16	Lateralization	O	O	
17	Morphology	C	C	C
18	Differentiation level	O		
19	pT	O/C*		
20	pN	O/C*		
21	pM	O/C*		
22	Degree of certainty	O	O	O
23	HPV **high risk** test results			C if HPV test performed
24	HPV **high risk** types detected			O
25	Nomenclature number(s)		O	O

O = optional ; C = compulsory ; HR : highly recommended if not feasible to deduce from other variables
O/C : compulsory if INSZ not delivered ; optional when INSZ is delivered
O/C* : only compulsory if TNM-classification applicable for the coded specimen
/// : *not applicable*

For details on the specific variables, see BCR-protocol (www.kankerregister.org or www.registreducancer.org).

INVITED LECTURES

L 43
How the registry data help in the research on the Belgian population…

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The link between the cytohistopathology registry and cancer screening programs in Belgium.

In Belgium, the population-based screening programs for cervical, colorectal and breast cancer, recommended by Europe, are organized at a regional level. In Flanders, a screening program has been set up for each of the three cancers, in Wallonia and Brussels for breast and colorectal cancer. Opportunistic screening also exists beside the organized screening programs. For cervical cancer, the screening test is a cervical smear followed by (a HPV-test for atypical cells and) a colposcopy with biopsy if indicated. A mammography is the primary screening test for breast cancer with additional imaging and possible sampling/biopsy procedures as follow-up investigation. For colorectal cancer screening, a positive faecal occult blood test (FOBT) test is followed by a colonoscopy with biopsy if indicated.

In this context, the Belgian Cancer Registry plays a pivotal role by collecting – regardless of the diagnosis and next to the cancer registration data – all anatomopathological test results of cervical-, breast-, and colorectal samples to constitute the central cyto-histopathology(CHP-) registry. Linked to the databases of screening programs, this CHP-registry and the cancer registration data allow to evaluate and monitor the quality of the three screening programs, to optimize the organization and also to perform research. Using these two population-based registries, the characteristics of patients and lesions can be described and compared between the participants and non-participants. The non-participants who underwent an opportunistic screening can be identified using the linkage with the database of the health insurances.

In the evaluation of screening programs, an important element is to determine interval cancers and to compare their characteristics with the screen-detected cancers and the cancer cases diagnosed in the non-participants. The interval cancer rate is one of the performance indicators of the EU Guidelines which allows the evaluation of the sensitivity of the screening programme. Identification of interval cancers facilitates to research the amount of 'not detected cancers' by the primary screening instrument as well as by the follow-up. To further differentiate, analyze and characterize true and missed interval cancers, radiological review projects are currently ongoing for the breast cancer screening.

CHP-based research also allows to explore the screening programs by analyzing for example the participation and the coverage but also to monitor the screening tests by

calculating the sensitivity, specificity and positive predictive value. Also the evaluation of the follow-up investigations following a positive screening test is profoundly based on the CHP-registry in combination with nomenclature data from the health insurances. This research includes the type of follow-up examinations, delay to follow-up and results of follow-up.

In the context of cervical cancer screening, the additional registration of HPV-test results and the detected HPV-types in the cervical CHP-registry provide information to compare the HPV-screening with classical smear-screening. It also enables the study of the distribution of the various HPV-types. These analyses will be extended by linkage with HPV-vaccination data.

The calculation of the colorectal adenoma detection rate is an important indicator in colorectal screening to evaluate the quality of colonoscopies. The linkage of the colorectal CHP-registry with a future colonoscopy-registry will thus allow a thorough monitoring of the quality of colonoscopies.

Cancer registration allows to describe cancer incidence and spatiotemporal trends. Since the goal of the screening programs is to detect and treat pre-malignant lesions, it is expected to observe a fall in the colorectal-, cervical- and breast cancer high stage cancer incidence during the course of the screening programs. A screening program should ultimately lead to a decrease in mortality.

Cancer Registry

The Belgian Cancer Registry (BCR) is in charge of producing and monitoring the Belgian statistics on cancer incidence (incl. spatiotemporal trends), prevalence and survival.
The Health Law of December 2006 (1) provides a legal basis for the Cancer Registry and describes the clinical and pathological anatomy pathway for data collection. Data are routinely gathered in these two settings allowing most of first-way missed cases to be declared by the other one. The law also provides the authorisation to use the national number (social security number INSZ-NISS) for the patient identification. The use of this unique number puts a new perspective on linkage with other available medical and/or administrative data (e.g. nomenclature and pharmacological data) and hence longitudinal research. Such a linkage not only requires the authorization of the Privacy Commission but also implies severe measures and rules for privacy protection and confidentiality.
The Flemish region achieved a full coverage and completeness since the year of incidence 1999. The Flemish data were published in 'Cancer Incidence in Five Continents', volume VIII and IX (2). From 2004 on, data are complete for the whole country: they were recently published in 'Cancer Incidence in Five Continents', volume X(3). Data are now available for 8 consecutive incidence years, 2004-2011 (4); cancer incidence data 2012 will be published in November 2014.
A first survival report (diagnosis 2004-2008) for Belgium and the three regions was published in December 2012 (5), followed by a specific booklet in 2013 on incidence and

survival of Childhood Cancer (2004-2009) (6). In June 2014, a prevalence report (1, 5, 10, 15 and 20 years) was made available for the first time (7).
The progress made during the last years, is clearly related to the legislation activities, new initiatives on clinical registration in the Flemish, Brussels and Walloon hospitals, and the sustained registration efforts of the pathology laboratories.
Although incidence, survival and prevalence figures represent a very important output of a cancer registry, this can only be considered as a first deliverable in a multi-step process. Cancer registries indeed see their role more and more extended in cancer control (8).
The increasing initiatives of the clinicians and pathologists in quality of care studies, the introduction of a national cancer plan and the active involvement of patient organisations demonstrate a growing interest in cancer control. Cancer control not only aims to reduce the incidence, morbidity, and mortality of cancer but also wants to improve the quality of life of cancer patients through the systematic implementation of evidence-based interventions in prevention, early diagnosis, treatment, and palliative care. In these different domains, several projects have already lead to a wide range of results and are still ongoing.
Quality of care studies fit in this concept and should result in optimizing treatment strategies, reducing variability in treatment and improving the prognosis of cancer patients. These studies mostly focus on process, structure and outcome parameters.
In a European and International context, the Belgian Cancer Registry participates in Eurocourse, Eurocare, EPAAC, Rarecarenet, Cancer Incidence in Five Continents (IARC) and the Concord study.

1 Wet houdende diverse bepalingen betreffende gezondheid van 13 december 2006, artikel 39. Belgisch Staatsblad, 22 december 2006. Loi portant dispositons diverses en matière de santé du 13 décembre 2006, article 39. Moniteur Belge, 22 décembre 2006
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4 Cancer Incidence in Belgium, 2011, Belgian Cancer Registry, Brussels, 2013
5 Cancer Survival in Belgium 2004-2008, Belgian Cancer Registry, Brussels, 2012
6 Cancer in Children and Adolescents 2004-2009, Belgian Cancer Registry, Brussels, 2013
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8 Armstrong BK The role of the cancer registry in cancer control. Cancer Causes Control 1992;3:569-579

L 44
Pigmented lesions state of the art.

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Histopathology is the current gold standard for the diagnostic classification of melanocytic tumors. However, a subset of these neoplasms cannot be unequivocally separated into benign (naevi) and malignant (melanoma) categories. Examples of borderline lesions with intermediate histological features include atypical Spitz tumour, pigmented epithelioid melanocytoma and lesions that may occur in congenital melanocytic naevus particularly proliferating nodules. Over the past two decades, progress has been made in uncovering genetic alterations in melanocytic neoplasia. Techniques, such as comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH), have demonstrated that melanomas and benign naevi differ at the chromosomal level. A recurrent pattern of chromosomal aberration in melanoma including copy-number gains at 6p, 1q, 7q, 8q, 17q, and 20q as well as losses at 9p, 9q, 10q, 10p, 6q and 11q has been described (Am J Pathol 2003, 163:1765–1770). Positive aCGH findings have been identified in 92% -95% (J Mol Diagn 2013, 15: 581-591; Am J Pathol 2003, 163:1765–1770) of melanomas. Benign naevi, by contrast, typically lack chromosomal copy-number aberrations and are diploid with the exception of some subsets of Spitz naevi, which may have copy-number increases of the entire short arm of chromosome 11 as the only chromosomal aberration (usually those with a desmoplastic phenotype) (Am. J. Pathol 2000, 157:967–72). Although, frequent chromosomal aberrations in atypical nodular proliferations arising in congenital naevi have been described, these abnormalities differ from those seen in melanoma in the type of aberrations. In the former there are gains and losses of multiple entire chromosomes, rather than the copy-number changes involving chromosome fragments that are commonly observed in the latter. Also the chromosomes involved are usually different: (osses of chromosome 7 in atypical nodular proliferations, and frequent losses of chromosomes 9 and 10 in melanoma (Am J Pathol 2002, 161:1163–1169).

Using data derived from CGH to identify ideal probe targets, several FISH assays have been developed recently to aid in the diagnosis of melanocytic neoplasms. An algorithm-using signal counts from a combination of 4 probes targeting chromosome 6p25 (RREB1), 6 centromere, 6q23 (MYB), and 11q13 (CCND1) provided the highest diagnostic discrimination. This algorithm correctly classified melanoma with 86.7% sensitivity and 95.4% specificity (Am J Surg Pathol 2009, 33:1146-1156). Subsequent similar studies have reported a comparably high sensitivity and specificity in the distinction of melanoma from naevi (Arch Dermatol 2010, 146:273–278; Mod Pathol 2011, 24:613–623; J Mol Diagn 2013, 15: 581-591). In addition potential applications of FISH for solving a variety of diagnostic dilemmas in the evaluation of melanocytic

tumours, including differentiating blue nevus–like metastasis from blue nevus, mitotically active nevus from nevoid melanoma, dysplastic nevi from superficial spreading melanoma and evaluation of pigmented spindle cell melanocytic tumours have been described (Am J Surg Pathol 2010, 34(6):816–821; Am J Surg Pathol. 2009, 33:1396–1400; Am J Surg Pathol 2009, 33:1783–1788; Am J Surg Pathol 2011, 35:1733–1742).

In a series of 27 histologically ambiguous melanocytic tumours with long-term clinical follow up (at least 5 years, or with metastasis), the test correctly predicted 100% of cases (6/6) that later metastasized (Am J Surg Pathol 2009, 33(8): 1146-1156). It should be noted, however, that 6 additional ambiguous melanocytic tumours which, did not metastasize (with follow up time ranging from 6.5 to 10 years), were also positive with the melanoma FISH test. Other series that have evaluated the FISH technique in ambiguous melanocytic tumours, using clinical behaviour as the gold standard, showed lower sensitivity (50-60%) and specificity (50-87%) (Modern Pathology 2010, 23: 413–419; Modern Pathology 2011, 24: 613–623). Nonetheless, the European study led by Vergier found that FISH analysis improved the sensitivity and specificity of diagnosis as compared with expert opinion alone. It should be mentioned that criteria used by Gaicer, et al never reached a validation stage and there was considerable inter-observer disagreement (19% to 25%) in the interpretation of the FISH analysis, probably related to selection bias of the nuclei counted. To increase the sensitivity to detect clonal chromosomal imbalances, representative areas of the entire tumour should be visually inspected for abnormal FISH signals for each probe and areas with the most abnormal FISH signals should then be counted (Am J Surg Pathol 2014; 38: 824–831). Experience of FISH testing of 804 ambiguous melanocytic lesions performed as part of routine workup at University of California, San Francisco has been reported. Of the 630 cases that tested negative by FISH, the final diagnosis was benign in 489 (78%) cases, ambiguous in 91 cases (14%), and malignant in 50 cases (8%). A positive FISH result was observed in 124 cases, with a final diagnosis of melanoma in 117 (94%). The authors concluded that FISH testing can help reduce the number of equivocal diagnoses in ambiguous melanocytic neoplasms, in particular if FISH testing is positive (Am J Surg Pathol 2014; 38: 824–831).

Gerami et al, have identified 9p21 (CDKN2A), 6p25 (RREB1), 11q13 (CCND1), and 8q24 (MYC) as a 4-new probe set with improved discriminatory power in differentiating melanomas from naevi (sensitivity of 94% and specificity of 98% in a validation set of a mixed group of melanomas and melanocytic naevi) (Am J Surg Pathol 2012;36:808–817). In addition, this probe set significantly decreased susceptibility to false positives as a result of tetraploidy. The inclusion of 9p21 in a melanoma FISH assay is particularly helpful in lesions with spitzoid morphology (Am J Surg Pathol 2012;36:81–88) and related with prognosis. Patients with atypical Spitz tumours (AST) and heterozygous 9p21 deletion are a subset of neoplasm with overall excellent prognosis compared with ASTs with homozygous deletions (Am J Surg Pathol 2014;38:638–645). Cases with 6p25 or 11q13 gains also have higher risk for aggressive clinical behavior than FISH-negative ASTs or cases with 6q23 deletions (Am J Surg Pathol 2013, 37:676–684).

It is becoming increasingly clear that Spitz tumours are a heterogeneous group of genetically and biologically distinct lesions. In addition to initially described cases with mutations of HRAS, typically accompanied by copy-number increases of the entire short arm of chromosome 11 as the only chromosomal aberration (Am. J. Pathol 2000 157:967–72); ASTs with a combination of BRAFV600E mutations and biallelic loss of the tumor suppresor BAP1 on chromosomal 3p21 have been characterized (Am J Surg Pathol 2012, 36(6): 818–830). Such tumours are seen in the context of a familial syndrome in which patients present with multiple cutaneous Spitzoid lesions and have a family history of uveal melanoma. Further associations include cutaneous melanoma, mesothelioma and other solid organ tumours. Spitzoid lesions present as intradermal, often dome-shaped or polypoid, and vertically oriented proliferations of large epithelioid melanocytes arranged in large cellular aggregates, often in continuity with a conventional acquired naevus. Presence of a lymphocytic infiltrate targeting the larger spitzoid cells, overlapping with that which has been previously reported as Spitz naevus with a halo reaction is another morphological feature (Am J Surg Pathol 2014, 38:1088–1095). Identical sporadic spitzoid tumours also associated with loss of BAP1 and BRAFV600E mutations have also been described.
Recent studies have revealed a high frequency of rearrangements of kinases in the remainder Spitz tumours. Rearrangements resulting in fusion kinases of ROS1, ALK, RET, NTRK1, and BRAF were observed in 55% of Spitz nevi, 56% of ASTs, and 39% of spitzoid melanomas (Nat Commun. 2014; 5: 3116). Spitz naevi/tumours with ALK rearrangement are usually compound proliferations with a predominant intradermal growth, characteristic plexiform morphology lack of pigmentation (15/17) (Am J Surg Pathol 2014;38:925–933).

Although morphologic analysis of melanocytic tumors still is the gold standard in melanoma diagnosis, molecular analysis is becoming a useful adjunct in histological ambiguous melanocytic tumours. In light of the benefits and limitations of molecular analysis as an ancillary tool in melanoma diagnosis, the pathological report needs to be carefully worded to ensure that the limitations of these tests are clearly comprehensible.

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FREE PAPER SESSION

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ORAL PRESENTATIONS

ORAL PRESENTATIONS

O1

Case Report

AUTOPSY CASE REPORT: INTRA-EPITHELIAL LYMPHOCYTES, VILLOUS ATROPHY, FOAMY MACROPHAGES AND BEYOND...

E. Bodson[1], S. Verschuere[2], G. Vanneste[3], L. Libbrecht[2], M. Praet[2], K. Geboes[2]
[1] AZ Sint Jan, Brugge; [2] UZ Gent; [3] AZ Groeninge, Kortrijk

Content :

A female 51 year old female patient died after a chronic and debilitating illnes. A diagnosis of Hepatitis C was made 19 years before. Six years later, she displayed unexplained chylous ascites and pleural effusions and associated mild hypoproteinemia. Nephropathy, tumoral pathology, restrictive cardiomyopathy and lymphatic pathology (obstruction or congenital dysfunction) were excluded. Investigation for a granulomateous disease or hematologic malignancy was negative. The patient later presented a trapped right lung and eventually developed cachexia due to progressive malabsorption. Duodenal biopsies one year before death revealed atrophic villi and increased intra-epithelial lymphocytes, but celiac disease was thought to be very unlikely based on serology. Biopsies of the oesophagus, stomach and colon were unremarkable. The patient died after developing multiple chronic infections and respiratory insufficiency.

Autopsy material from the colon revealed a pseudomembranous colitis. The liver showed limited microvesicular steatosis. Duodenal samples showed autolysis of the mucosa, and large collections of foamy histiocytes in the submucosa . PAS and Ziehl-Neelsen stain were negative. Deeply situated submucosal macrophages showed brown pigmentation and a similar pigmentation was found in the smooth muscle cells of the muscularis propria (onfirmed by TEM). The pigment was shown to be lipofusin deposition.

This image is known as brown bowel syndrome (BBS), a rare disease with only 27 scientific reports in the literature. The term is derived from the orange brown discoloration of the bowel wall, caused by lipofuscin deposits. They can also occur in apical enterocytes, but usually only in the smooth muscle cells of the muscularis propria. BBS is considered to be the result of Vitamin E deficiency. Such a deficiency can be the result of a deficient enteral uptake of fat soluble tocopherol in several malabsorption syndromes such as celiac disease and even obesity surgery.. Vitamin E serves as a membrane protector. Low levels can cause damage to the phosholipid layer in membranes leading to deposits. In the present case, the malabsorption leading to BBS was most likely due to a lysosomal storage disorder, which was responsible for the foamy macrophages in the submucosa and confirmed by TEM. Since no fresh material or remaning blood was available, the exact nature of this storage disease remains however unresolved.

BBS is thought to cause a smooth muscle cell mitochondrial myopathy: The pigment deposits are situated mostly perinuclear and ultrastructurally they can be found within a single unit membrane. Over time the loss of mitochondria can lead to atrophy and atonia of the muscle wall, and neuronal functional disorders, which may give rise to dystrophia, weight loss, protein deficiency edema and motility problems.

O2
IGG4 EXPRESSION IN LABIAL SALIVARY GLANDS IN PATIENTS WITH SICCA SYNDROME

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Introduction:
Sjögren's syndrome is an autoimmune disease mainly characterized by a sicca syndrome resulting from destruction of lacrimal and salivary glands. Mikulicz's disease (MD), formerly considered as a subtype of Sjögren's syndrome, is now recognized as a manifestation of IgG4-related disease (IgG4-RD). Our objective is to study IgG4 tissue expression in minor labial salivary glands from patients suffering from sicca syndrome in our hospital from 1998 to 2012 and further identify cases corresponding to the diagnostic criteria of IgG4-related autoimmune sialoadenitis (MD).

Material and methods:
Minor labial salivary glands biopsies corresponding to grade 3 or 4 of Chisholm and Mason classification were included (≥ 50 infiltrating cells per field). We performed IgG4 and total IgG immunostainings. We applied currently recommended histological diagnostic criteria for IgG4-related autoimmune sialoadenitis (MD) requiring a number of IgG4-positive plasma cells per high power field (HPF), first one > 50 and second one > 100 and a ratio of IgG4+/IgG+ positive cells > 40%. Clinical and biological features of these patients were also collected.

Results:
Among 993 salivary gland biopsies, 263 corresponded to the inclusion criteria.
Only 42 of them expressed IgG4 but the number of IgG4 + cells per HPF was < 50 in each case. None of each tested biopsy reached the ratio IgG4+/IgG+ plasma cells >40%.
In the group of patients with IgG4 + biopsies (n=42) relative to the group of patients with IgG4 –biopsies (n=221), we identified a significantly higher proportion of patients with secondary Sjögren's syndrome (21% vs 7%). Moreover, patients in IgG4 + group more frequently arthralgia (62% vs 41%, p=0.017), positivity of ANAs (69% vs. 47%, p=0.0116), total IgG level >1.5 g/dL (29% vs 12%, p=0.0153) and ESR >20 mm/h (42% vs 19%, p=0.0012).

Conclusion:
In our cohort, no IgG4-related autoimmune sialoadenitis (MD) case was detected. However, IgG4 immunostaining resulted in the identification of two patient subgroups. The higher proportion of IgG4+ plasma cells in biopsies from secondary Sjögren's syndrome may suggest common pathophysiological pathways with IgG4-RD.

O3
IMPACT OF NEOADJUVANT THERAPY ON CANCER-ASSOCIATED FIBROBLASTS IN RECTAL CANCER

Laurine Verset[1], Joke Tommelein[2], Xavier Moles Lopez[3], Christine Decaestecker[3], Tom Boterberg[2], Isabelle Salmon[1,3], Marc Mareel[2], Marc Bracke[2], Olivier De Wever[2]*, Pieter Demetter[1]*

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Introduction:
Cancer-associated fibroblasts (CAFs), also called myofibroblasts, are increasingly recognized as promoters of tumor progression. It is poorly investigated whether cancer management protocols, such as neoadjuvant radio(chemo)therapy, have an impact on CAFs and, by consequence, on tumor progression.

Aim :
This prompted us to study the impact of neoadjuvant radio(chemo)therapy on the α-SMA/epithelial area ratio in rectal cancer, and the impact of this ratio on recurrence-free survival.

Methods:
Immunohistochemistry for the CAF marker α-SMA and the proliferation marker Ki67 was performed on sections from 98 rectal cancers of which 62 had undergone neoadjuvant radio(chemo)therapy.

Results:
Computer-assisted quantitative analysis showed that the α-SMA/epithelial area ratio was higher after neoadjuvant therapy, and that rectal cancers with high α-SMA/epithelial area ratio had low proliferation rates. Interestingly, the α-SMA/epithelial area ratio was an adverse prognostic factor with regard to recurrence-free survival in univariate analysis.

Conclusion:
These results suggest that neoadjuvant treatment has an impact on CAFs in primary rectal cancer. The correlation of CAFS with decreased recurrence-free survival and abundant experimental data in the literature suggest that under certain circumstances, not yet very well understood, CAFs may favor tumor progression. Our observations may explain why better local control fails to be translated into longer recurrence-free survival.

O4
EXPRESSION OF MAP KINASES IN PAPILLARY THYROID CARCINOMA

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Introduction:
Papillary thyroid cancer (PTC) frequently carries several genetic alterations in genes coding for proteins that activate the mitogen-activated protein kinases (MAPK) signalling pathway, important in the regulation of cell growth, differentiation, and survival.

Aim:
The present study aims to investigate transduction cascades of MAPK signalling pathway [extracellular-regulated kinase (ERK), Jun N-terminal Kinase (JNK) and p38] involved in tumorigenesis of PTC.

Methods:
Twenty cases of PTC and its variants (eleven cases of classic PTC and nine cases of follicular variant of PTC) were retrieved from the biobank. Immunohistochemical analysis of total and phosphorylated forms of ERK, JNK and p38 was performed. Frozen sections were then solubilized to perform Western blots of total and phosphorylated MAP kinases in PTC and its variant.
Effect of MAPK inhibitors U0126 (ERK inhibitor), SP 600125 (JNK inhibitor) and SB 203580 (p38 inhibitor) are being analysed on BC-PAP, TPC-1, and WR082-W-1 cell lines by MTT assay and Western blots.

Results:
ERK activation was seen as nuclear immunolabelling in 15/ 20 PTC cases in less than 10 % of tumour cells. JNK activation was seen in 7/20 cases in less than 10 % of tumour cells as nuclear and membranous immunolabeling. However, total ERK and total JNK was seen as nuclear and cytoplasmic immunolabelling in 10/20 and 16/20 cases respectively in greater than 50% of tumor cells. p38 MAPK phosphorylation was seen as abundant nuclear and cytoplasmic immunolabelling in 11/ 20 PTC cases (6 cases of follicular variant of PTC and 5 cases of classic PTC). Total p38 was immunolabelled in 14/20 cases of PTC. A one way ANOVA test showed significant difference between the ERK, JNK and p38 phosphorylation ($p<0.01$).
By Western blotting, phosphorytated p38, phosphorylated ERK and JNK were detected in 17, 7 and 10 cases respectively. The signals were much stronger in the follicular variant of PTC as compared to classic PTC cases. p38 was also detected in normal thyroid tissue whereas the phosphorylated form of p38 was not detected in the normal thyroid tissue. MTT assay showed a decrease in the number of viable cells after culturing with

the p38 and ERK inhibitor in all the cell lines. Western blot analysis revealed decreased phosphorylation of p38 and ERK after treating the cell lines with inhibitors.

Conclusion:
Increased expression and activation of p38-MAPK cascade in PTC and its variants indicate that it is functional in PTC. These data suggest that p38 is activated in a larger proportion of PTC than ERK or JNK. The molecular profiling of PTC could reveal the altered biological pathways involved in the genesis of this common endocrine malignancy.

O5
PARACRINE REGULATION OF STROMAL PROTEIN EXPRESSION IN BREAST AND COLORECTAL CANCER

M. Van Bockstal [1], K. Lambein [1], M. Van Gele [1], G. Braems [1], V. Cocquyt [1], H. Denys [1], M. Bracke [1], L. Libbrecht [1], O. De Wever [1]
[1] UZ Gent, Gent

Introduction:
The significance of the tumor microenvironment in cancer progression is increasingly acknowledged. We recently observed that reduced periductal decorin expression correlated with the presence of myxoid stroma in ductal carcinoma in situ (DCIS) of the breast. Both reduced decorin expression and myxoid stroma were significantly associated with an increased recurrence risk in DCIS. We hypothesized that suppression of stromal decorin might contribute to the pathogenesis of myxoid stroma, as decorin plays an important role in collagen fibrillogenesis. Myxoid stroma and reduced stromal decorin expression may reflect the propensity of some DCIS lesions to progress to invasive cancer.

Aim:
We aimed to investigate paracrine regulation of decorin expression and related extracellular matrix proteins in the tumor microenvironment. This may enable us to identify the composition of a tumor-promoting and invasion-permissive stroma.

Methods:
Immortalized CT5.3-hTERT colon-cancer associated fibroblasts were used as an in vitro model. The effects of cytokines on stromal protein expression were assessed by RT-PCR and Western blotting. Co-culture experiments were performed with conditioned medium (CM) of breast and colorectal cancer cells. Immunohistochemical analysis was carried out on DCIS specimens. Adhesion assays were performed with breast and colorectal cancer cells on decorin and collagen coatings, and on fibroblast-derived matrices.

Results:
TGFβ and bFGF suppressed decorin expression in fibroblasts, although these cytokines differentially regulate α-SMA expression. RT-PCR analysis revealed that TGFβ strongly enhanced the expression of biglycan, versican and collagen (COL1A1) in fibroblasts, whereas it downregulated decorin, lumican and fibromodulin. This was confirmed at the protein level by Western blotting. Despite the previously reported significant correlation between myxoid stroma and reduced stromal decorin expression, no statistically significant association was found between myxoid stroma and periductal biglycan or versican expression, although there was a tendency for the latter. Co-culture

experiments with breast cancer cell-derived CM enhanced expression of versican and biglycan, and caused downregulation of decorin in fibroblasts. Treatment of fibroblasts with colorectal cancer cell-derived CM resulted in upregulation of versican, biglycan and decorin expression. At present we cannot explain this discrepant response. Adhesion assays revealed that decorin coatings prevent adhesion of breast and colorectal cancer cells, which might explain the downregulation of decorin in invasion-permissive peritumoural stroma. When cancer cells are seeded on matrices derived from TGFβ-treated fibroblasts, they adhere in a highly organized manner along preformed 'tracks', compared with the random adhesion pattern on matrices derived from untreated fibroblasts.

Conclusion:
TGFβ seems to be a powerful modulator of the peritumoural extracellular matrix. In the breast, a decorin-depleted stroma enriched in versican might represent a tumor-induced invasion-permissive extracellular matrix. Further investigations on larger cohorts are required to determine whether changes in stromal protein expression are associated with poor outcome in breast and colorectal carcinoma.

O6

Case Report

CASE REPORT: RARE SOFT PLAQUE DISEASE CAN MYSTIFY STAGING OF COLON CARCINOMA.

E. Bodson[(1)]
[1] AZ Sint Jan, Brugge

Content:
A 55-year old female patient was diagnosed with a rectosigmoidal carcinoma.

Endoscopy showed a circumferential tumour in the rectosigmoid confirmed as being carcinoma on biopsy. A CT scan showed a diffuse tumoural transformation of the lower pelvis and a segmental widening of the bowel wall over 10 cms, with a possible extension into the posterior bladder.

A total mesorectal excision (TME) for a cT4 rectal carcinoma was planned, without neo-adjuvant therapy. The rectum was resected with the cervix and part of the vagina attached. During surgery the posterior wall of the bladder showed invasion by tumour and a partial cystectomy was also performed.

Macroscopically, the TME resection specimen showed plaques of extensive yellowish pinkish friable tissue on the ventral, dorsal, and bilateral circumferential surfaces, surrounding the tumour. The tumour measured 7 cm longitudinally, and was circular. There was extension into the mesorectal fat. After fixation, the friable plaques were revealed as grey tumour masses, which were different from the tumour, showing a white-yellowish fat alteration at the mesorectum.

Microscopically, a moderately differentiated adenocarcinoma was found, consisting of focally cribriform neoplastic glands, with a characteristic dirty necrosis. Four of 26 lymph nodes were invaded by carcinoma. The plaques featured a population of dyscohesive normotypical epithelioid cells with a deeply eosinophilic granular cytoplasm, and abundant small and large caliber blood vessels. There were interspersed neutrophilic granulocytes and collections of plasma cells. This population was also abundantly present the partial cystectomy specimen, where no adenocarcinoma was found. The cells were negative for S100 and pan-cytokeratin and positive for CD 68 on immunohistochemistry. Ziehl-Neelsen revealed no Mycobacterium avium.

A von Kossa stain illustrated the presence of Michaëlis-Gutman bodies, which were not conspicuous at the haematoxylin-eosin stained slides.

The colon carcinoma was staged as a pT3 pN2a LV1, with transection only to the ventral

cut surface, cranial to the cervical fragment. Tumoural invasion into the cervix could not be demonstrated.

The plaques were diagnosed as Malakoplakia. It is a rare disease, featuring collections of macrophages with a pink granular cytoplasm. Underlying is a phagolysosome defect. They thus accumulate bacteria or undigested bacterial material in the phagolysosome.

Malakoplakia can be associated with colon carcinoma and E. Coli cystitis, both present in our patient.

Pathological differential diagnoses include Mycobacterium avium infection, granular cell tumor, Whipple's disease, or dyscohesive carcinoma.

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P1

Case Report

SUCCESSFUL NEOADJUVANT BRAF TARGETED THERAPY OF AN INITIALLY UNRESECTABLE BRAF-V600E MUTANT MELANOMA.

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[1] Hôpital Erasme, Bruxelles ; [2] UZ Brussel, Brussel

Content:
Successful neoadjuvant BRAF targeted therapy of an initially unresectable BRAF-V600E mutant melanoma

Clinical case
A 62-year old woman presented to our dermatology department because of a large pigmented lesion with multiple other adjacent smaller lesions on the internal side of her left ankle.

Physical examination confirmed a large pigmented skin tumour with irregular borders, surrounded by numerous satellite lesions. No other lesions or distant metastases were found. A biopsy was performed for pathologic diagnosis and for BRAF V600E mutation status.

Pathological analysis of specimen revealed a proliferation of large atypical melanocytic tumour cells in the superficial dermis. The tumours cells were positive for Melan-A and S100 by immunohistochemistry confirming the diagnosis of “in-transit” melanoma metastasis.

The BRAF mutation status was assessed by qPCR: a V600E mutation was found in the BRAF gene.

Given the extension of the lesion a neoadjuvant therapy with a BRAF-inhibitor therapy (vemurafenib) was preferred over surgery.

Ten weeks after the initiation of treatment, two new skin lesions located on the left upper arm and forearm were resected and the histopathology analysis revealed classical squamous cell carcinomas, secondary to the vemurafenib treatment. Treatment was changed to dabrafenib plus trametinib.

After 19 weeks of treatment, physical examination showed reduction in the thickness of the primary lesion. The satellite lesions had become non-palpable and there were no new distant metastasis. A surgical excision of the entire lesion was thus performed.

The microscopic examination of the primary lesion and of the satellite lesions revealed an abundant accumulation of pigmented macrophages, fibrosis and numerous often hyperplastic vessels.

Only very few melanocytic tumor cells positive for S-100 but negative for Melan-A remained in the dermis and in the dermo-epidermal junction of the primary lesion.

Discussion

Neoadjuvant therapy with a BRAF inhibitor represents a new therapeutic option for patients with BRAF-V600E-mutant locally advanced melanoma. So far only few case reports have been published on the use of anti-BRAF-therapy in the neoadjuvant setting for patients where complete surgical resection was considered impossible at diagnosis. As in our case, the pathological response to neoadjuvant BRAF-inhibition described in these case reports was almost complete in every patient. Regressed lesions are characterized by foamy histocytes and tumoural necrosis but pigmented macrophages or hyperplastic vessels were not previously reported.

The presence of an abundant vasculature with hyperplastic vessels in our case merits consideration as it resembles the phenotype described in Braf -/- mice with the presence of an increased number of endothelial precursor cells and dramatically enlarged blood vessels.

Conclusion

This case report illustrates a successful neoadjuvant BRAF-targeted therapy in a patient with locally advanced BRAF V600E mutant melanoma with a pathological response of more than 95% of tumor regression . This case supports the further investigation of neoadjuvant treatment with BRAF inhibitors in locally advanced BRAF V600E mutant melanoma.

P2

Case Report

ENDOMETRIOïD INVASIVE CARCINOMA WITH MUCINOUS DIFFERENTIATION SHOWING BOTH FGFR2 AND TP53 MUTATIONS BY NEXT GENERATION SEQUENCING.

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Content:

Recent data have allowed to suggest that targeted Next Generation Sequencing (NGS) system could be a useful tool to distinguish the two types of endometrial carcinomas (EC), in association with other pathological features (eg classical histology, presence of intraepithelial endometrial neoplasia (EIN), immunohistochemistry against PTEN, p53 and Beta-catenin,..) Traditionally, EC have been classified as type I and type II. Type I EC classically develop in the setting of hyperoestrogenism and frequently coexists with EIN. The prototypes of this type are endometrioïd and mucinous carcinomas. They are generally moderately or highly differentiated and have a favourable outcome.
The common molecular alterations described are microsatellite instability and mutations in the PTEN, PIK3CA, K-RAS, FGFR2 and CTNNB1 (Beta-catenin) genes. In particular, mutations in the fibroblast growth factor receptor 2 (FGFR2) tyrosine kinase genes have been identified mostly in type I endometrioïd carcinoma, in about 10% of cases.
By opposition, types II EC are oestrogen independent, associated with endometrial atrophy, generally poorly differentiated and associated with a less favourable outcome. They are typically associated with the mutations of the TP53 genes in particular in those with serous differentiation (90% of cases). However, some data also described a higher frequency of TP53 mutations in up to 30% of grade III endometrioïd carcinoma.

We describe here the case of a grade III endometrioïd carcinoma with partial mucinous differentiation occurring in a 74-year-old female and which was stadified pT1aN0 (the lesion infiltrated less than 50% of the myometrium). Adjacent to the invasive component, EIN lesions were observed. Mutational analysis of selected regions of 22 cancer-associated genes using NGS (Ion Torrent Personal Genome Machine) revealed mutations in TP53 and FGFR2.

Our case suggests that a small part of EC can share at least partially both type I and II EC mutational pattern, as described in the literature. Therefore, mutational genome sequencing status by the NGS could be a useful adjunct to classical histology and immunohistochemistry, to accurately classify EC, indicating distinct genetic pathways. This could help discover potential target therapy such as FGFR2 inhibitor for example.

P3

Case Report

CARDIAC FIBROSIS IN A PATIENT WITH CARDIAC SARCOIDOSIS: IN VIVO BIOPSY AND POST-MORTEM EXAMINATION.

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[1] CHU Brugmann Site Victor Horta, Bruxelles

Content:

A 61-year-old man with history of dual-chamber pacemaker implantation for third-degree atrio-ventricular block was admitted for invalidating exertional dyspnea. Echocardiography showed a cardiac failure with a 35% left ventricular ejection, while coronography was normal. Right ventricular endomyocardial biopsy revealed multiple non necrotising granulomas in the myocardia and the diagnosis of cardiac sarcoidosis was proposed. 18FDG positron-emission tomography also supported this diagnosis, as it showed intense captations in the heart and in the right lung.

In addition to classical heart failure therapy, an empirical treatment for cardiac and pulmonary sarcoidosis (prednisolone - 64mg) was administrated to this patient. Following lung abnormalities improvement without cardiac recovery, cyclophosphamide was added to the therapy and a cardioverter/defibrillator was implanted in this patient. Unfortunately, he ultimately developed a leucopenia complicated by pulmonary infections (pseudomonas aeruginosa and aspergillosis) culminating in septic shock. An autopsy was performed, that reported images of renal and hepatic shock, images of immunosuppression (lack of lymphocytes in the spleen), and images of pulmonary aspergillosis.

In the heart, the lungs and in the hilar lymph nodes, it reported large cicatricial areas corresponding to extensive cicatricial fibrosis, especially in the heart. Some fibrosis areas were even nodular, while no granuloma was seen.

By comparing the results of the *in vivo* endomyocardial biopsy with post-mortem examination, we suggest that the multiple non necrotising granulomas had been replaced by nodular fibrosis areas, following treatment.

This case is interesting as unrelenting evolution of sarcoidosis under immunosuppressive therapy occurs in only one-third of patients. Moreover, as the mortality associated with sarcoidosis is less than 5%, comparing in vivo biopsy and post-mortem examination is unusual. In addition, endomyocardial biopsy in sarcoidosis is only contributive in less than 20% because cardiac involvement tends to be patchy, and granulomas are more likely to be located in the left ventricle and basal ventricular septum than in the right ventricle, where endomyocardial biopsies are usually performed.

This case also illustrates the importance of the knowledge of the patient's history when interpreting *post-mortem* examination.

P4
A YOUNG PATIENT WITH A PERSISTENT CERVICAL ADENOPATHY, DIFFERENTIAL DIAGNOSIS.

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Aim
This case illustrates the difficulty of the differential diagnosis of a cervical adenopathy in a young patient without any particular medical history.

Methods and results
A 32-years old woman with a painless cervical adenopathy, without loss of weight, since a few weeks. An echography was performed and showed a necrotic center. The patient has positive serology for CMV (IgG and IgM) plaiting for a recent infection. Because of the persistence of the adenopathy since 1 year a biopsy was performed. This showed the presence of neoplastic cells with vesicular nuclei and prominent nucleoli. These cells were positive for CD45, CD20, Bcl2, CK19 and pancytokeratin. These cells were negative for CD45, CD30, CD15 and EBV. We discuss the possibility of a diffuse large B-cell lymphoma of an undifferentiated nasopharynx carcinoma. The molecular biology does not show a monoclonal population B. The final diagnosis was thus an undifferentiated carcinoma of the nasopharynx.

Conclusions
Undifferentiated carcinoma of the nasopharynx is rather rare in Europa, frequent in North-Africa and the Maghreb. In the populations at high risk, the patients are generally more than 30 year old, with a peak of incidence between 10 and 60 year and a sex ratio M/F of 3/1. The virus of Epstein- Barr (EBV) is associated almost always (whatever is the ethnic origin) and is considered as the main oncogenic agent. The diagnosis is often more difficult in the undifferentiated forms. The main differential diagnosis is the lymphoma (immunoblastic type). The distinction between these 2 entities may be very difficult on the only HES, for this reason is the roll of immunohistochemistry and molecular biology very important.

P5

Case Report

INTRAUTERINE UNEXPECTED DEATH AT 39 WEEKS GESTATIONAL AGE:A HISTORY OF PLACENTA.

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Content:
Intrauterine unexpected death at 39 weeks gestational age: a history of placenta.

We describe a case of 31-year old patient, gravida 2, para 1, with an uncomplicated pregnancy, presenting to the emergency ward at 40 weeks of gestation because of diminished foetal movements. The cardiotocogram as well as abdominal foetal ultrasonography showed no foetal heart rate and the intrauterine foetal death was confirmed. Three prenatal ultrasonographies realised in 1st, 2nd and 3rd trimester did not reveal any foetal or placental malformation. The amniocentesis revealed, surprisingly, bloody amniotic fluid. The research of foetal erythrocytes in maternal blood was negative as well as all haematological and bacterial screening of the mother. The autopsy of the foetus revealed pale skin colour and a Meckel's diverticulum. The placental examination showed a furcate insertion of the umbilical cord with one vessel ruptured. We conclude that the death was caused by foetal haemorrhage into the amniotic cavity following this vessel rupture. The insertion abnormality of the umbilical cord accompanied by a vessel rupture is a rare condition but represents a placental cause of an abrupt and unpredicted foetal death.

P6
CHOLESTERYL ESTER STORAGE DISEASE: AN UNDERRECOGNIZED CAUSE OF MICROVESICULAR STEATOSIS.

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Content:
A 33-year-old woman was referred to our institution because of a 6 month-biologic hepatic cytolysis. She presented with asthenia, emesis, diarrhea and several episodes of ENT infection (sinusitis) with pyrexia two weeks ago. Her medical history included hypercholesterolemia and urticaria.

Biological work-up revealed increased levels of GPT (57 U/l; N<34U/l), total cholesterol (296 mg/dl) and LDL-cholesterol (232mg/dl) with a decrease of HDL-cholesterol level (33 mg/dl). A liver biopsy showed microvesicular steatosis with discrete septal fibrosis. Taking into account this microvesicular steatosis, the elevated level of LDL-cholesterol with a low level of HDL-cholesterol and the absence of any apparent cause of hepatic cytolysis, a lysosomal acid lipase (LAL) deficiency was suspected. The level of LAL activity was measured using a dried blood spot and confirmed a deficiency.

Cholesteryl ester storage disease (CESD) is an underdiagnosed, autosomal recessive, progressive metabolic liver disorder caused by a variety of mutations of the LIPA gene. These cause reduced activity of LAL, which results in accumulation of cholesteryl esters in lysosomes. The entity is characterized by hepatomegaly, splenomegaly and extrahepatic manifestations like epigastric pain, emesis, anemia, malabsorption and steatorrhea. Onset of clinical manifestations can be present from the first year of life into adulthood. Differential diagnoses are non-alcoholic steatohepatitis, non-alcoholic fatty liver disease and cryptogenic liver disease. Statins don't reverse the disease manifestations, finally leading to liver failure.

Identification of this rare disorder is difficult. Characteristic histological findings include microvesicular steatosis, leading to fibrosis and micronodular cirrhosis. A diagnosis of CESD should be considered in any case of otherwise unexplained microvesicular steatosis.

P7

HISTOLOGICAL STUDY AND ANALYSIS OF VASCULAR CALCIFICATIONS IN PERITONEAL BIOPSIES: ADIPOCYTES AS NEW PLAYERS IN ENCAPSULATING PERITONEAL SCLEROSIS?

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Introduction :
Encapsulating peritoneal sclerosis (EPS) is a serious complication of peritoneal dialysis (PD). Besides the mesothelio-to-mesenchymal transdifferentiation (MMT) process, adipocytes were proposed recently to be involved in EPS.

Aim :
We tried to observe the origin of fibrosis in EPS

Methods :
We approached both hypotheses studying tissue expression of AE1/AE3 (mesothelial cells), calretinin (adipocyte marker), α-smooth muscle actin [α-SMA] (mesenchymal cells) in peritoneal biopsies from 3 patients suffering from EPS. Moreover, we assessed the interstitial inflammation and neoangiogenesis (CD3, CD4, CD8, CD20, CD68 and CD31 immunostainings, respectively) and the mineral composition of vascular calcifications (infrared microspectroscopy).

Results :
Three patients (1M/2F; age 17, 64 and 39 years, respectively) developed EPS after 21, 90 and 164 months of PD therapy. Mesothelial cells expressed AE1/AE3 and calretinin in controls but this expression disappeared and there was no migration of mesothelial cell into the interstitium in all EPS patients. In comparison with normal tissue biopsy, the adipose tissue with adipocytes physiologically calretinine+ was replaced by severe sub-mesothelial fibrosis. In these areas besides αSMA+ cells as expected we found a huge number of calretinin+ fusiform interstitial cells. We observed a neoangiogenesis in all EPS patients but we found vascular calcifications only in 1 patient (8 PD-related bacterial peritonitis) which contained mainly carbapatite.

Conclusion :
Conclusion: Taking into account the small number of studied cases, our results suggest that: (1) the involvement of MMT in peritoneal fibrosis is still unproven, (2) resident adipocytes represent probably underestimated sources of peritoneal fibroblasts and (3) the infrared microspectroscopy of tissue calcifications could be helpful to study the link between peritoneal infections and peritoneal vasculopathy in EPS.

P8

CLINICAL VALIDATION OF TARGETED NEXT GENERATION SEQUENCING FOR COLON AND LUNG CANCERS.

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Introduction :
Clinical validation of targeted next generation sequencing for colon and lung cancers. Introduction : Recently, next generation sequencing (NGS) has begun to supplant other technologies for gene panel sequencing which is now required for targeted therapies. However, transfer of NGS technology to an ISO15189-certified laboratory requires validation.

Aim :
Evaluation of the clinical applicability of targeted next generation sequencing for screening lung and colorectal cancers

Methods :
We validated the Ion Torrent AmpliSeq colon/lung cancer panel interrogating 1850 hotspots in 22 genes using the Ion Torrent Personal Genome Machine. First, we used commercial reference standards carrying mutations at defined allelic frequency (AF). Then, 51 colorectal adenocarcinomas (CRC) and 39 non small cell lung carcinomas (NSCLC) were retrospectively analyzed.

Results :
Sensitivity and accuracy for detecting variants at an AF >4% was 100% for commercial reference standards. Among the 90 cases, 89 (98.9%) were successfully sequenced. 83/86 samples were concordant between NGS and the reference test; i.e. KRAS for CRC and EGFR for NSCLC, with the 3 discordant cases each characterized by an AF <10%.

Conclusion :
Overall, the AmpliSeq colon/lung cancer panel was specific and sensitive for mutation analysis of gene panels and can be incorporated into an ISO15189-certified laboratory.

P9

Case Report

AIDS-RELATED CEREBRAL HISTOPLASMOSIS, A CASE REPORT.

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Content :
AIDS-related cerebral histoplasmosis, a case report

We describe the case of a 32 year-old Italian woman who presented at the emergency ward with a third episode of generalized tonic clonic seizure in the last twenty-four hours.

Anamnesis revealed an abortion 6 months ago and several journeys to South East Asia about ten years ago.

The initial brain MRI displayed a 13mm left frontal lesion, ring enhancing, surrounded by edema and showing no restricted diffusion.

The initial blood analysis highlighted a lymphopenia with a CD4 count of 71 cells/mm^3 (N: 388-1841), and CRP reaching 14mg/dL. HIV serology was positive for a type 1 HIV with a 5 339 429/mL viral load. Anti-toxoplasma IgG rate was at 21.6 UI/mL revealing previous infection.

The diagnosis of AIDS-related cerebral toxoplasmosis has been proposed and a treatment was initiated, based on an ART association and an empirical therapy against Toxoplasma Gondi (pyrimethamine and sulfadiazine).

After six weeks, the control MRI did not demonstrate any significant evolution of the intracranial lesion, raising the possibility of differential etiological diagnosis and motivating a cerebral biopsy.

Direct examination in Microbiology demonstrated small (3-5µm) intracellular, oval, thin-walled yeast.

Histopathological assessment revealed a brain parenchyma infiltrated with plasmocytes and macrophages. Higher magnification showed that the cytoplasm of macrophages was filled with round to oval aggregated yeast-like cells.

The microbiology laboratory confirmed by culture the diagnosis of *Histoplasma var. capsulatum*, and a treatment by Amphotericin B followed by Itraconazole was initiated. Histoplasmosis is a telluric dimorphic fungus that has been reported from every continent except Antarctica. It is endemic to North and South America, South-East Asia, South Africa and Northern Italy. Transmission occurs through inhalation of spores when contaminated soil is disrupted; there is no human to human transmission.

After inhalation the fungus spread through the reticulo-endothelial system. In 90% of the cases the infection is silent and histoplasmosis can remain latent for years. It can also cause pneumonitis or disseminated infection, either following primary infection or reactivation of latent histoplasmosis.

Immunosuppressed patients tend to develop severe symptoms and HIV patients generally are symptomatic when CD4 count drop under 150 cells/mL.

In our case the infection was limited to the central nervous system which is a rare clinical presentation in immunocompromised patient. Indeed, they are more likely to exhibit either lung-limited or disseminated infection. In case of disseminated presentation, CNS involvement occurs in 5 to 20% of cases.

P10
MARSH CRITERIA VALIDITY IN CHILDHOOD CELIAC DISEASE DIAGNOSTIC.

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Objective
The differential diagnosis of atrophic villosity associated with lymphocytosis in pediatric samples remains a histological challenge. Here, we assess the diagnostic yield of Marsh criteria in samples from children with Celiac disease, Crohn disease or congenital IgA deficiency.

Methods
Thirty-six patients were selected by referent gastroenterologists. Among them, 32 and 4 present with clinical and/or biological signs of Celiac disease or Crohn, respectively. Among patients with Celiac disease, 5 suffer from congenital IgA deficiency. Duodenal biopsies were reviewed by 3 pathologists blinded for clinical data, using Marsh criteria. The diagnostic of Celiac disease was retained if biopsies were devoid of Brunner glands. Search for Giardia, bacterial colonies, granulomas and nodular lymphoid hyperplasia was performed. Reproducibility between pathologists and the clinic-pathological concordance were evaluated in each group.

Results
Pathological diagnostic of Celiac disease according to Marsh criteria was established in 14 cases among the group of 27 immunocompetent children. Atypical chronic bulbitis was observed in 9/27 cases. No Celiac disease was found among the patients with congenital IgA deficiency. Pathologists did not reach the same conclusions in six cases within Celiac group and 1 case in Crohn group. In all of these 7 cases, the final diagnostic was chronic bulbitis with or without villous atrophy.

Conclusions
Marsh criteria showed high specificity, but poor sensitivity, in diagnosing Celiac disease in immunocompetent children. The limited number of cases with congenital IgA deficiency hampers any significant conclusion.

	Celiac (-)	Celiac (+)	
Marsh (-)	5	13	
Marsh (+)	0	14	
	5	27	32

Sensibility = 14/27 = 51.8%
Specificity = 1- (0/5) = 100%

P11

Evaluation of a rapid, sensitive and fully automated Idylla™ BRAF Mutation Test* starting directly from FFPE samples.

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Introduction :
The annual incidence of malignant melanoma has increased dramatically over the past few decades. In the US, almost 80,000 new cases of melanoma are expected in 2014 resulting in almost 10,000 deaths. For patients with metastatic melanoma, BRAF-targeted therapies have demonstrated dramatic antitumor activity if the tumor cells carry characteristic V600 mutations in the BRAF gene.
Several molecular tests are available to analyze the BRAF tumor V600 mutation status. All are labor intensive and require trained personnel, while some lack sensitivity or miss some relevant mutations. We evaluated the performance of the IdyllaTM BRAF Mutation Test, a novel fully automated test that can define the BRAF V600 mutation status in formalin-fixed paraffin embedded (FFPE) tumor tissue with a short turnaround time of 90 minutes.

Material and Methods:
The IdyllaTM BRAF Mutation Test detects the V600E/E2/D and V600K/R/M mutations in FFPE tissue sections from human melanoma at high sensitivity.
The Test consists of a disposable Cartridge, which contains all reagents for fully integrated sample preparation and real-time PCR and is processed on the Idylla™ System. The mutation status is determined by three allele-specific duplex PCR reactions, designed to detect the BRAF Wild Type, V600E/E2/D and V600K/R/M mutations.
The Test takes about 90 minutes, starting from insertion of a FFPE tissue section into the Cartridge up to reporting of the test result. Hands-on time is less than 2 minutes and no specific molecular lab experience is needed.
In this evaluation, results of Idylla™ have been compared with the Roche Cobas® 4800 BRAF V600 Mutation Test. Analytical sensitivity, which is defined as the minimum number of cells per PCR to permit reliable detection of 1% V600E or 1% V600K, has been determined using commercially available FFPE reference material.

Results:
In total 15 melanoma samples were tested. For all samples, valid test results were obtained for both methods. In 13 (86.7 %) cases a concordant result was found.
One discordant sample yielded mutant call on Idylla™ (V600K/R/M) while Cobas detected no mutation. Idylla™ showed an increased analytical sensitivity (400 and 1000 cells for 1% V600E and V600K respectively) compared to the Cobas (3.9 ng ≈ 1000 cells

for 5% V600E) while time-to-result was reduced from 8 to 1.5 hrs. The other discordant sample yielded insufficient input call on Idylla™ while Cobas detected no mutation. Visual inspection of the block after the Idylla™ test, showed that there was only little tissue left, what could explain the insufficient input result on Idylla™.

Conclusion:
The IdyllaTM BRAF Mutation Test is a reliable, fully automated method for BRAF V600 mutation testing directly on FFPE tumor tissue with superior sensitivity, ease-of-use, and turnaround time compared to existing diagnostic tests.

P12

EVALUATION OF THE IDYLLA BRAF MUTATION ASSAY PERFORMANCE USING THE NOVEL FULLY-AUTOMATED IDYLLA™ SYSTEM.

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Background & aim:

Mutations in the BRAF gene are found in diverse cancers such as melanoma, papillary thyroid and colorectal cancers. Targeted therapy with BRAF and MEK inhibitors has the potential for rapid tumor regression in patients whose melanoma harbors a BRAF mutation. Tumor mutation status is usually assessed in formalin-fixed paraffin-embedded (FFPE) tissue scrapings. In our daily routine, the test involves shipment of the paraffin block from our local pathology laboratory to a dedicated molecular pathology center. The IdyllaTM system (Biocartis), which is a novel fully-automated molecular diagnostics platform, offers the possibility to perform the BRAF mutation analysis in our laboratory in a short time (90 min) and with minimal hands-on time (<2 min/FFPE curl). This study aims to evaluate the performance of the prototype IdyllaTM BRAF Mutation test (Biocartis) on FFPE specimens using the Idylla System.

Methods:

Two reference standards (Horizon Dx) and 19 FFPE samples from melanoma tumors, were subjected to the prototype Idylla BRAF Mutation test using the Idylla system. These FFPE samples were previously tested for V600 BRAF mutations by wild-type blocking real-time PCR followed by Sanger sequencing, allele-specific real-time PCR (Qiagen, in-house) or StripAssay (Viennalab). The tumor area and percentage tumor cells in the clinical samples ranged from 7 to 216 mm2 and from 40% to >95% tumor cells, respectively. If the sample contained less than 50% tumor cells, macro-dissection was performed to reach a content of at least 50% tumor cells. Single or multiple FFPE curls of 4 µM, 5 µM, 10 µM or 15 µM were inserted into the Idylla BRAF Mutation test cartridge. In case of discrepancy, the samples were further analyzed by digital droplet PCR (in-house).

Results:

The Idylla assay detected the BRAF mutant alleles which were present in the reference standards at a frequency of 5%. Results obtained by the Idylla assay and wild-type blocking PCR followed by Sanger sequencing were concordant in 8/9 (89%) samples. For one sample, the Idylla assay picked up another mutation, which was confirmed by digital droplet PCR. The concordance between the Idylla assay and allele-specific PCR or StripAssay was 90% (9/10). In one sample, no mutation was detected by allele-specific PCR, whereas a V600 mutation was found by both the Idylla assay and digital droplet PCR.

Conclusion:
The prototype Idylla BRAF Mutation test shows a very good concordance with wild-type blocking PCR followed by Sanger sequencing, allele-specific PCR or StripAssay. Our findings demonstrate that the Idylla system is a fast and reliable method to determine the BRAF mutation status in FFPE melanoma tumor samples. Therefore, this novel molecular diagnostics platform can easily be implemented in a clinical laboratory setting.

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