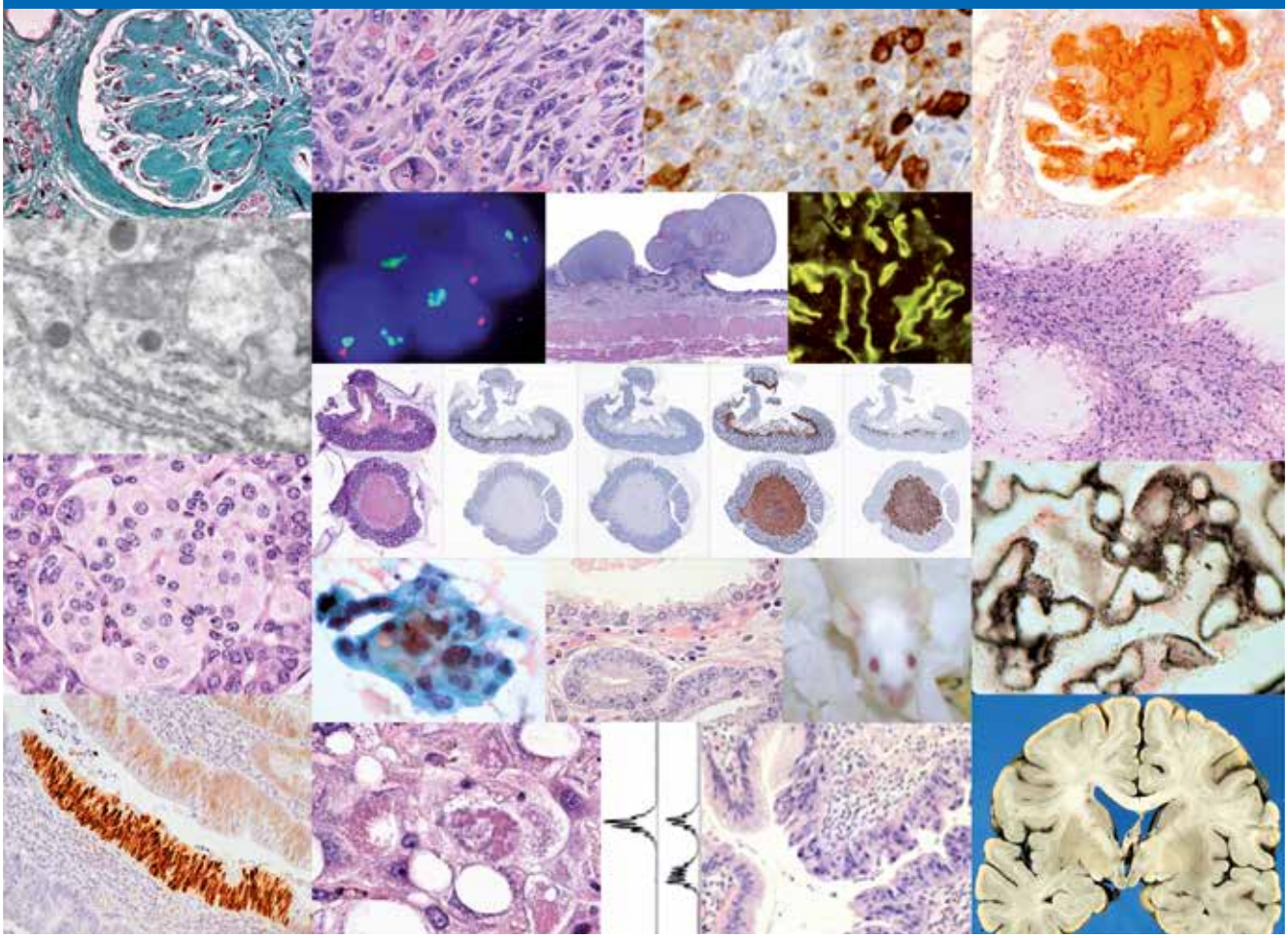
BWPath

BELGIAN WEEK OF PATHOLOGY

7th BELGIAN WEEK OF PATHOLOGY

OCTOBER 12-15, 2016

Congrescentrum Augustijnenklooster - Ghent



WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

www.belgian-society-pathology.eu

THE *THINPREP* SYSTEM

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- **Significantly more effective** in the detection of HSIL+ and LSIL than conventional Pap.[1*]
- **Superior sample quality** compared to conventional Pap.[1,2]
- **Adds flexibility** to your workflow to increase efficiency.
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MORE THAN 650 MILLION THINPREP PAP TESTS PERFORMED[3]

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* The ThinPrep 2000 System is significantly more effective than the conventional Pap smear for the detection of low-grade squamous intraepithelial (LSIL) and more severe lesions in a variety of patient populations.

† Assays not available in all countries. For information on specific products available for sale in a particular country, contact your sales representative.

THIS MATERIAL IS NOT FOR DISTRIBUTION IN THE U.S.

References: 1. ThinPrep 2000 System [package insert]. MAN-02060-002 Rev. 001. Marlborough, MA: Hologic, Inc.; 2011. **2.** Klinkhamer, et al. Liquid-based Cervical Cytology. *Cancer Cytopathol.* 2003;99(5):263-71. doi:10.1002/cncr.11673. **3.** Hologic, Inc. Data on file.

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WELCOME

Dear Colleagues,

The previous six editions of the Belgian Week of Pathology (BWP) were a huge success. Last year's edition took place a Thagaste – Trefpunt Augustijnen, a magnificent monastery at historical Ghent; based on your positive comments and the excellent possibilities for interactions with our sponsors, we decided to organise again at this unique location.

Pathology is currently undergoing important changes, and this evolution in our field has impact on medical decisions in daily practice. This prompted us to develop an up-to-date scientific program, accessible for experienced pathologists as well as for trainees and other young colleagues.

Internationally renowned experts and leading Belgian pathologists, molecular biologists and clinicians will share their insights with us in a constructive and friendly atmosphere. There will be ample time for more close contacts with them during coffee break, lunches and the Thursday night drink.

Traditionally we welcome our cytotechnologists on Saturday morning, and we developed a postgraduate course on Wednesday; together with the free paper session, this first day of the meeting offers an excellent opportunity for youngsters to gain more in-depth knowledge into our rapidly evolving medical specialty.

We would like to thank our partners from the industry on their renewed support! Some made interesting suggestions, and it is our pleasure to work together in such a constructive way.

We are delighted to see you at this Seventh Belgian Week of Pathology. Enjoy the meeting and the city!

Sincerely yours,

Pieter Demetter
BWP 2016 President

Advancing Cancer Diagnostics
Improving Lives

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Beginning to end integration

A holistic system-wide approach to the lab

Leica Biosystems is a global leader in workflow solutions and automation. As the only company to own the workflow from biopsy to diagnosis, our goal is to help you break down the barriers and strive towards providing patient diagnosis within a 24 hour period. Because time is of the essence, don't wait another day. Learn more at LeicaBiosystems.com

95.14422 Rev A - 09/2016

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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 16, 2016.
The result of evaluation was sent to the first authors on september 6th

- Oral presentations will be presented during the Free paper Session on Wednesday from 14:00 to 15:30.
- Poster presentations will take place during the morning and afternoon coffee breaks of the Wednesday 12 October.

Posters will be displayed from Wednesday to Saturday on the assigned boards 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 available

Hospital Sint Lucas : Groenebriel, 1 - 9000 Ghent

Hotels

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

Event Coordinator

DME Events
Anne-France De Meyer – 102, Av.Carsoel – 1180 Brussels – Belgium
Tel : +32 2 375 36 26 / E-mail : anne.france.de.meyer@dme-events.eu

Ghent Tourism Office

Botermarkt, 17A – 9000 Ghent
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BELGIAN SOCIETY OF PATHOLOGY

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President : Thomas Gevaert

BWP STEERING COMMITTEE

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Verbeke S.	Gand
Weynand B.	UZLeuven
Willocx F.	Brussels

PROGRAM OVERVIEW

		KLOOSTERGANGEN ROOM Ground floor	AUGUSTIN ROOM Ground floor – 200 pax	HIPPO/CARTAGHUE Ground floor – 120 pax
WEDNESDAY October 12	8:30 - 8:35	Exhibition Area Posters	Opening : P. Demetter	
	8:35 - 10:00	Exhibition Area Posters	Postgraduate Course Gynaecopathology	
	10:00 - 10:30	Coffee Break **Posters Session 1**		
	10:30 - 12:00	Exhibition Area Posters	Postgraduate Course Gynaecopathology	
	12:00 - 14:00	**LUNCH** Exhibition Area Posters		**Satellite Symposium MSD 12:00 – 12:45** **Satellite Symposium THERMOFISHER 13:15 – 14:00**
	14:00 - 15:30	Exhibition Area Posters		Free Paper Session
	15:30 - 16:00	Coffee Break **Posters Session 2**		
	16:00 - 17:30	Exhibition Area Posters	Postgraduate Course Gynaecopathology	
THURSDAY October 13	8:30 - 10:00	Exhibition Area Posters	9:00 - 10:00 What's new in Dermatopathology	8:30 - 10:00 Ethics and Economy: Legal issues in medical mistakes
	10:00 - 10:30	Coffee Break Exhibition Area Posters		
	10:30 - 12:00	Exhibition Area Posters	What's new in Dermatopathology	
	12:00 - 12:45	Exhibition Area Posters	**Keynote Lecture:** P. Hofman (Nice, France)	
	12:45 - 14:00	**LUNCH** Exhibition Area Posters	**Satellite Symposium SECTRA 13:15 – 14:00**	
	14:00- 15:30	Exhibition Area Posters	New WHO 2016 : Classification of Brain Tumours	Kidney and Bladder Tumours
	15:30 - 16:00	Coffee Break "Exhibition Area"		
	16:00 - 17:30	Exhibition Area Posters	New WHO 2016 : Classification of Brain Tumours	Kidney and Bladder Tumours
	17:30 - 18:30	**Drink, Cheese & Wine**		**Satellite Symposium TRIBVN/HAMAMATSU 17:30 – 18:15**

PROGRAM OVERVIEW

FRIADAY October 14

	KLOOSTERGANGEN ROOM Ground floor	AUGUSTIN ROOM Ground floor – 200 pax	HIPPO/CARTAGHUE Ground floor – 120 pax
9:00 - 10:30	Exhibition Area Posters	9:00 - 10:30 Pathology of Serosal Surfaces	9:30 - 10:30 Bone Pathology
10:30 - 11:00	Coffee Break Exhibition Area Posters		
11:00 - 12:00	Exhibition Area Posters	Pathology of Serosal Surfaces	Bone Pathology
12:00 - 13:00	Exhibition Area Posters	**Keynote Lecture :** R. Paï (Scottsdale, U.S.A.)	
13:00 - 14:00	**LUNCH** Exhibition Area Posters		**Satellite Symposium ASTRAZENECA 13:15 – 14:00**
14:00 - 15:30	Exhibition Area Posters	Pathology of the Digestive Tract	
15:30 - 16:00	Coffee Break Exhibition Area / Posters		
16:00 - 16:45	Exhibition Area Posters	Pathology of the Digestive Tract	
16:45 - 17:00		**BWP 2016 Awards :** Boël Prize / Best Poster	
17:00 - 18:00		**Satellite Symposium MULTIPLICOM 17:00 – 17:45**	General Assembly Belgian Society of Pathology

SATURDAY October 15

9:00 - 10:30	Exhibition Area	Surgical Pathology: Selected topics	Program for Cytotechnologists
10:30 - 11:00	Coffee Break Exhibition Area		
11:00 - 12:30	Exhibition Area	Surgical Pathology: Selected topics	Program for Cytotechnologists
12:30 - 12:35		Closing: P. Demetter	
12:35-14:00	**LUNCH** Exhibition Area Posters		

WEDNESDAY
THURSDAY
FRIDAY
SATURDAY

Belgian Week of Pathology 2016 12 – 15 October 2016 Ghent, Belgium

MSD

Satellite Symposium on PD-L1 Testing in NSCLC

Wednesday, 12 October 2016

12:00 –12:45

Invitation

Congress Center Augustijnenklooster
Augustin Room
Ghent, Belgium

CHAIR

Prof. Dr. Birgit Weynand
Head of Pathology
University Hospital of Leuven
Leuven, Belgium

AGENDA

12:00 – 12:05	**Welcome and Introduction**	Prof. Dr. Birgit Weynand
12:05 – 12:20	**Relevance of PD-L1 Testing in Non-Small Cell Lung Cancer**	**Pulmonologist View** Prof. Dr. Johan Vansteenkiste
12:20 – 12:35	**Feedback from the experience on PD-L1 Testing & Scoring**	**Pathologist View** Prof. Dr. Nicky D'Haene
12:35 – 12:45	**Questions and Discussion**	All Participants

MSD Oncology

WEDNESDAY 12 MORNING

Room Augustin

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

08:35-12:00: **Postgraduate Course Gynaecopathology:**
Update in vulvar pathology.
Chairpersons: J.-C. Noël (Brussels), P. Delvenne (Liège)

08:35 ***Inflammatory lesions and melanocytic tumours.***
A. Theunis (Brussels)

09:15 ***Soft tissue tumours.***
N. De Saint Aubain (Brussels)

10:00-10:30 ***Coffee break*** */ Poster Session 1*

P7 Immunohistochemical expression study of ATRX, an ALT suppressor protein, in small cell lung cancer.
M. Vanhooren / UZ Brussel

P8 Immunomorphology of Ewing's and Osteosarcoma.
M. Vanhooren / UZ Brussel

P9 PD-L1 expression in human atherosclerotic disease: of any importance?
R. Forsyth / UZ Brussel

P10 Osteolysis in non-sarcomatous metastatic bone lesions: 'graft versus host'?
R. Forsyth / UZ Brussel

10:30 ***Epithelial tumours.***
J.-C. Noël (Brussels)

11:15 ***Slide seminar.***
S. Franke (Liège)

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

WEDNESDAY 12 AFTERNOON

Room Hippo

12:15-12:45 Satellite Symposium MSD

13:15-14:00 Satellite Symposium THERMOFISHER

14:00-15:30 **Free paper session**
Chairpersons: P. Demetter (Brussels), C. Cuvelier (Ghent)

14:00 **P1** UCA1 Overexpression Is a New Independent Prognostic Marker in Bladder Cancer Associated with Increased Patient Survival.
L. Lebrun / ULB Erasme

14:15 **P2** Vascular impact on dementia: The role of cerebral amyloid angiopathy and microinfarction on the development of dementia.
D. Thal / UZ Leuven

14:30 **P3** Vascular Insulin-like Growth Factor Receptor Type 2 (IGF2R) Expression is Upregulated in Malignant Tumours.
A.-L. Trépant / ULB Erasme

14:45 **P4** Use of immunohistochemistry and next generation sequencing for the classification of glioblastomas.
C. Delancre / ULB Erasme

15:00 **P5** Immunohistochemical expression study of ATRX, an ALT suppressor protein, in invasive breast cancer, ductal type.
H. Leus / UZ Brussel

15:15 **P6** Clinical application of targeted next generation sequencing for colorectal cancer patients: a multicentric Belgian experience.
Q. Fontanges / ULB Erasme

WEDNESDAY 12 AFTERNOON

15:30-16:00 ***Coffee break*** */ Poster Session 2*

P11 PD-L1 expression in Giant Cell Tumour of Bone: new treatment options beyond the osteo-immunological system?
R. Forsyth / UZ Brussel

P12 Case Report: TWO PATIENTS UNDERGOING SURGERY FOR OESOPHAGOGASTRIC JUNCTION'S ADENOCARCINOMA WITH UNEXPECTED FINAL DIAGNOSIS.
Koopmansch C. / ULB Erasme

P13 A unusual bile duct tumour in a patient with bilateral adrenal gland Hyperplasia.
Dehon R. / UCL Saint-Luc

P14 A young woman with a thoracic low-grade fibromyxoid sarcoma.
Bienfait L. / ULB Erasme

Room Augustin

16:00-17:30 **Postgraduate Course Gynaecopathology:**
non-tumoural endometrial Pathology.
Chairpersons: J.-C. Noël (Brussels), C. Bourgain (Bonheiden)

16:00 **Normal cycling endometrium and metaplasia.**
E. Marbaix (Brussels)

16:40 **Dysfunctional proliferative and secretory endometrial disorders and dysfunctional uterine bleedings.**
C. Bourgain (Bonheiden)

17:30 **End of the day**

THURSDAY 13 MORNING

Room Hippo

08:30-12:00 **Ethics and Economy: legal issues in medical mistakes.**
Chairpersons: M. Lammens (Antwerp), K. Cokelaere (Ieper)

08:30 **Evaluation of potential medical mistakes by pathologists: the Belgian Procedure.**
E. Marbaix (Brussels)

09:00 **The Belgian Order of Physicians: advising, supporting, sanctioning.**
L. Thienpont ((Belgian Order of Physicians, Provincial Council of East-Flanders)

09:30 **Medical mistakes by pathologists: the juristic point of view in Belgium.**
Ph. De Smet (Brussels)

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

10:30 **Towards a model proposal for service level agreement for second opinion in pathology.**
S. Callens (Leuven)

11:00 **Procedures in case of medical mistakes by pathologists: experiences from The Netherlands.**
J. Broekman (Den Bosch, The Netherlands)

11:30 **Tips and tricks in case of self-suspicion of medical error.**
I. Michielsens (Antwerp)

Room Augustin

09:00-12:00 **What's new in dermatopathology?**
Chairpersons: A. Theunis (Brussels), S. De Schepper (Ghent)

09:00 **What's new and relevant in epidermal tumours?**
M. Fernandez-Figueras (Barcelona, Spain)

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

10:30 **Ex vivo dermoscopy and derm dotting.**
M. Haspeslagh (Ardooie)

11:00 **Case presentations**

12:00-12:45 **Keynote lecture :**
Chairperson: I. Salmon (Brussels)

The role of the pathologist in the era of liquid biopsies.
P. Hofman (Nice, France)

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

SECTRA

Knowledge and passion

5 Key criteria for evaluating Digital Pathology

The adoption of digital pathology is evolving and offers functionality that goes far beyond the microscope. These new opportunities significantly increase workflow efficiency. They move time-consuming tasks to the computer and allow the pathologist to spend more time on reviewing cases. Here are five key criteria when evaluating a solution for digital pathology.

1 Optimized workflow

Access to all relevant patient data in one workstation.

Minimum mouse mileage and clicks through seamless integration of control and interface.

High-speed image display through web technology and serverside rendering.

Improved ergonomics, avoiding shoulder and neck problems.

2 Collaboration with other specialists

Easily share information across department boundaries.

Tailored dynamic worklists and support for multi-disciplinary team meetings.

Sharing of workload and second opinions.

Strategy towards integrated diagnostics.

3 Availability anytime, anywhere

View, present and discuss from any workstation.

Vendor Neutral Archive (VNA) for centralized storage.

VNA

Scalable to handle growth of users and production.

4 More consistent reviews

Automated image analysis for frequent cases.

Support for counting and percentage calculations.

Teaching functionality with easy tag and search.

Compare with patient history data.

5 Integration with healthcare IT solutions

Support for standards like HL7 and DICOM to integrate with EMRs, LIS, etc.

HL7 DICOM EMR

Vendor agnostic approach.

Part of the full enterprise image management strategy.

Presentation: How to create a business case for digital pathology?

When: Thursday 13th of October at 13:15
Where: Augustin Room
Presentor: Stijn Rabaey, Account Manager Belgium & Luxemburg

THURSDAY 13 AFTERNOON

Room Augustin

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

14:00-17:30 **New WHO 2016 classification of brain tumours.**
Chairpersons: I. Salmon (Brussels), M. Lammens (Antwerp)

14:00 **Novelties in the WHO classification of brain tumours.**
D. Figarella-Branger (Marseille, France)

14:45 **Molecular aspects of glioblastoma.**
M. Le Mercier (Brussels)

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

16:00 **Update on pituitary tumours.**
B. Lopes (Charlottesville, U.S.A.)

16:45 **Slide seminar**

Room Hippo

14:00-17:30 **Kidney and bladder tumours.**
Chairpersons: T. Gevaert (Antwerp), S. Aydin (Brussels)

14:00 **A urologist's point of view: importance of good pathology reporting on clinical urology practice.**
S. Joniau (Leuven)

14:45 **Molecular diagnostics in kidney cancer.**
A. Lopez-Beltran (Lisbon, Portugal)

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

16:00 **New and emerging entities in kidney cancer.**
R. Montironi (Ancona, Italy)

16:45 **Novelties from the new WHO-release and integration of molecular data in bladder cancer diagnostics.**
Y. Allory (Créteil, France)

17:30-18:15 **Satellite Symposium TRIBVN/HAMAMATSU**

17:30-19:00 **Drink, Cheese & Wine**

7th Belgian Week of Pathology 2016

Congrescentrum Augustijnenklooster Academiestraat 1, 9000 Ghent, BELGIUM

Satellite symposium introduced by Hamamatsu & TRIBVN Healthcare teams

HAMAMATSU
PHOTON IS OUR BUSINESS

TRIBVN
HEALTHCARE

FRIDAY 14 AFTERNOON

Room Augustin

9:00-12:00: **Pathology of serosal surfaces.**
Chairpersons: A. Hoorens (Brussels), I. Salmon (Brussels)

09:00 **Serosal cytology: morphological aspects.**
F. Thivolet-Beju (Lyon, France)

09:30 **Immunohistochemical panel and differential diagnoses in pleura and Peritoneum.**
M. Remmelink (Brussels) and K. Dhaene (Aalst)

10:00 **Molecular aspects of mesotheliomas: NGS and FISH.**
P. Pauwels (Antwerp) and N. D'Haene (Brussels)

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

11:00 **Mucinous and pseudo-mucinous lesions of the peritoneum.**
J. Misdraji (Boston, U.S.A.)

11:30 **The Belgian mesothelioma registry.**
M. Praet (Ghent) and M. Rosskamp (Brussels)

Room Hippo

09:00-10:30 **Bone pathology.**
Chairpersons: S. Verbeke (Gand), C. Galant (Brussels)

09:30 **Giant cell tumours.**
R. Forsyth (Brussels)

10:00 **Bone forming lesions.**
R. Sciot (Leuven)

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

11:00 **Vascular and notochordal lesions.**
C. Bouvier (Marseille, France)

11:30 **Cartilaginous tumours.**
J. Bovée (Leiden, The Netherlands)

Room Augustin

12:00-13:00: **Keynote lecture**
Chairperson: A. Jouret-Mourin (Brussels)

A practical approach to small bowel biopsy interpretation: coeliac disease and its mimics.
R. Pai (Scottsdale, U.S.A.)

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

FRIDAY

Belgian Week of Pathology

AstraZeneca Satellite Symposium

Friday 14th October 2016,

13h15 – 14h00

Room Augustin, Augustijnenklooster Ghent

Liquid or solid: What biopsy to use for determining EGFR T790M mutations in lung cancer patients?

Liquid biopsy is the next big evolution in molecular testing. With the expected availability of Tagrisso® (Osimertinib) for NSCLC patients progressing on first line EGFR therapy with a T790M mutation the liquid biopsy will have to find its place in clinical routine.

The reason to test: clinical data of Osimertinib
Professor Veerle Surmont, Pneumology department, UZ Gent

Realising the full potential of liquid biopsy for EGFR T790M testing
Professor Patrick Pauwels, Pathology department, UZ Antwerpen

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BELGIAN WEEK OF PATHOLOGY

FRIDAY 14 AFTERNOON

Room Hippo

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

Room Augustin

14:00-16:45 **Pathology of the digestive tract.**
Chairpersons: A. Hoorens (Ghent) , N. D'Haene (Brussels)

14:00 **Lymphomatoid polypoid lesions of the digestive tract.**
A. Camboni (Brussels)

14:45 **Tumour regression grading in digestive tumours.**
R. Langer (Bern, Switzerland)

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

16:00 **Role of RAS and BRAF analysis and MMR immunohistochemistry in treatment decisions in colorectal cancer.**
M. De Man (Ghent)

16:45-17:00 **BWP 2016 Awards:**
Boël Prize
Best Poster

17:00-17:45 **Satellite Symposium MULTIPLICOM**

Room Hippo

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

18:00 **End of the day.**

FRIDAY

human onco prenatal

7th Belgian week of Pathologie

Visit our **Symposium**

BRCAness in ovarium cancer

Friday 14 October 2016 from 16.45-17.30h

Multiplicom Enabling personalized medicine

Come & visit us at our booth **C1** Follow us:

SATURDAY 15 AFTERNOON

Room Hippo

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

09:00 **Colposcopy: the clinical correlation between cytology, HPV, biopsy and visual assessment.**
P. De Sutter (Brussels)

09:45 **Cervical cytology: (ab)normal and some rarities.**
T. Ruitenbeek (Groningen, The Netherlands)

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

11:00 **Slide seminar**

Room Augustin

09:00-12:30 **Surgical pathology: selected topics**
Chairpersons: A. Jouret-Mourin (Brussels), N. D'Haene (Brussels)

09:00 **Classification and pathology reporting of appendiceal mucinous neoplasms and appendiceal carcinomas.**
J. Misdraji (Boston, U.S.A.)

09:45 **IgG4 related diseases.**
M. Komuta (Brussels)

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

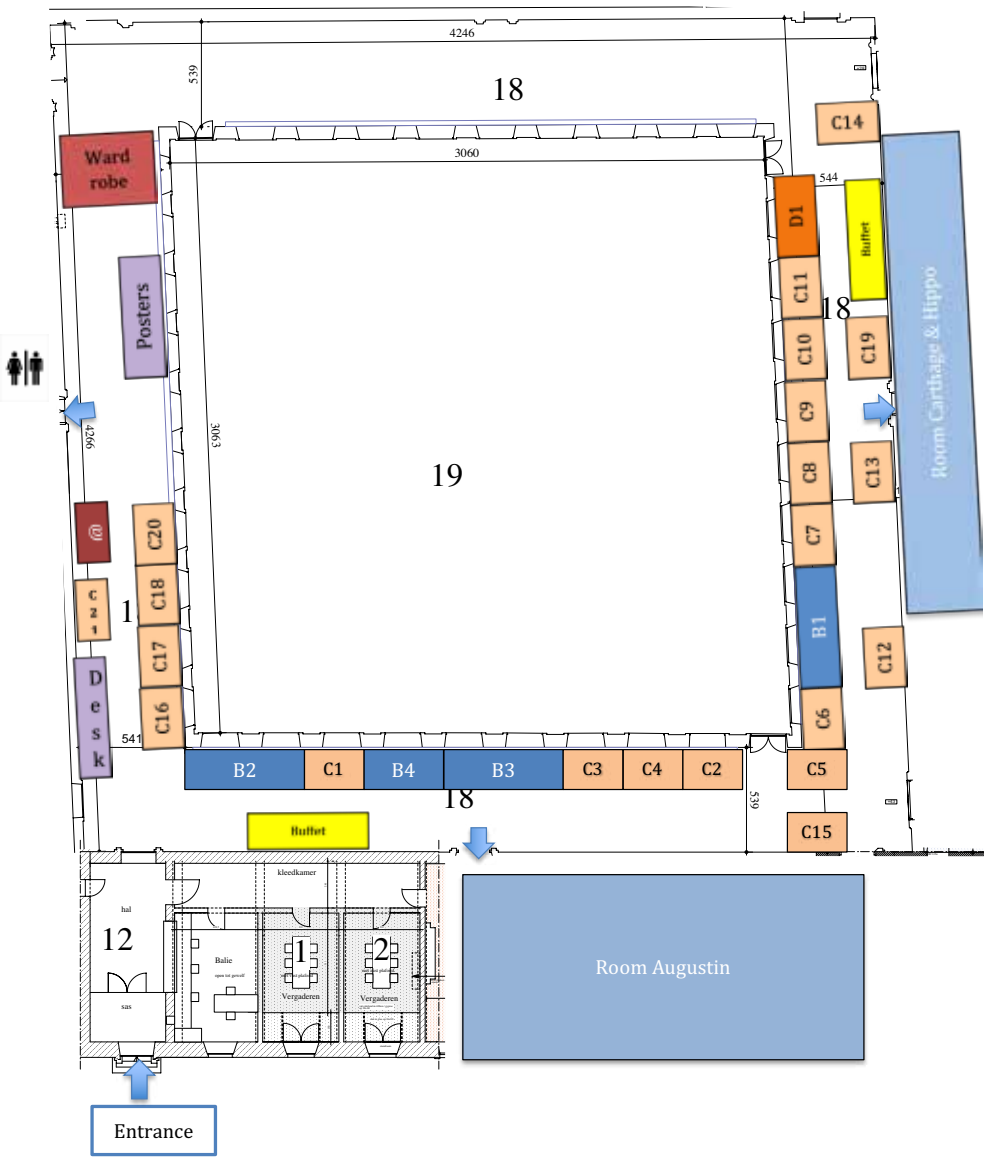
11:00 **Tumour budding in the digestive tract.**
I. Zlobec (Bern, Switzerland)

11:45 **Carcinoma of unknown primary: from immunohistochemistry to molecular profiling.**
K. Oien (Glasgow, U.K.)

12:30-12:35 **Closing of the BWP 2016**
P. Demetter (Brussels)

12:35-14:00 **Lunch**

EXHIBITION FLOOR



Catering		
		BUFFETS
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INVITED LECTURES

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INVITED LECTURES

L 1

INFLAMMATORY LESIONS AND MELANOCYTIC TUMOURS.

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Inflammatory lesions

Introduction
Accurate diagnosis of vulvar inflammatory lesions can sometimes be reached on histological examination alone but good clinical-histological confrontation is mandatory in many cases. Ideally clinical data must encompass: age of the patient (ie LCH nap children), duration, evolution, location, morphology of the lesions and symptoms (ie recurrent similar lesion at the same place: Fixed drug eruption: medication? HSV: vesicular and painful?...). To mention presence of cutaneous, buccal or ocular lesions could be useful (ie Bechet disease: buccal lesions?). Relevant cutaneous and internal diseases history can also helpful (ie: IBD: metastatic Crohn disease?, anorexia and carential diseases...). Some inflammatory pathologies have a high predilection for the anogenital areas and are often associated with buccal involvement. Others dermatosis are seen on vulva as elsewhere on the skin with similar histological pictures. It should be noted that normal variants exist (vestibular papillomatosis, sebaceous gland hyperplasia. And finally, in any cases performing PAS is never unnecessary.

Zoon's vulvitis
Zoon's vulvitis is very less common that Zoon's balanitis. It is characterized by a dermal quite dense band infiltration of plasma cells. Extravasated erythrocytes, lymphocytes and rarely neutrophils may be present. Perls coloration to highlight hemosiderin can help to make the diagnosis. The granular layer is thin or absent and keratinocytes are lozenge-shape. Spongiosis could be seen.

Spongiotic Dermatosis
Eczema: allergic, irritative, seborrheic dermatitis (with infant variant: "Napkin dermatitis" and rarely the ulcerated infantile granuloma). Eosinophilic spongiosis with some dyskeratotic cells are the hallmarks of incontinentia pigmenti-IP (stage 1, from birth to 3 months age), disease that can be sometimes only located on the vulva.

Psoriasiform Dermatosis
Flexural psoriasis: psoriasiform pattern less typical than in classical psoriasis and associated spongiosis. Diseases that closely mimic psoriasis: Dermatomycosis, syphilis. Psoriasiform dermatitis with epidermal pallor should "ring the bell" of the nutritional deficiencies (pellagra, acrodermatitis enteropathica) and necrolytic migratory erythema (glucagonoma syndrome).

Vesiculobullous Dermatosis
Subepidermal autoimmune blistering diseases are usually disseminated and can involve the vulva. The histology is the same as that seen elsewhere. The point is to obtain a biopsy from perilesional area to ovoid picture of non-specific ulceration and a biopsy for direct immunofluorescence-DIF (in physiological serum or "Milieu de Michel").

Acantholytic Dermatosis
With dyskeratosis: Hailey-Hailey disease (genital, flexural and friction areas), genital papular acantholytic dyskeratosis which is characterized by papules and nodules on genital skin (rare, spectrum of Darier / Hailey-Hailey disease?). In these pathologies DIF is negative. *Without dyskeratosis:* Pemphigus vulgaris-PV often involve buccal and genital areas (in contrast of superficial pemphigus) and Pemphigus vegetans have a predilection for genital and flexural zones. Suprabasal acantholysis is often subtle and masked by pseudoepitheliomatous epidermal hyperplasia with microabscesses of eosinophils. DIF is positive.

Lichenoïd Dermatosis
Lichen planus (and his erosive variant) is usually generalized but can be only seen in vulva. Lichen sclerosus (LS) is usually located on anogenital areas but disseminated lesions can occur (white spot disease). It should be noted that vulvar location of vitiligo is typical in children and can be considered in the differential diagnosis of early LS. LS can be the substrate of dysplasia, SCC and rarely melanoma. Fixed drug eruptions (sometimes bullous) have a predilection for anogenital area. Stevens-Johnson involve usually both genital and buccal mucosa.

Non infectious Granuloma
Metastatic Crohn's disease: non caseating granulomas associated with perivascular mixed infiltrate or granulomatous vasculitis.

Pitfalls
Langerhans cell: Lanbgerhans cell histiocytosis (LCH) is usually a disseminated disease with frequent anogenital area involvement. In infants the diaper area is typically affected with a seborrheic-like dermatitis aspect. Differential diagnosis, sometimes challenging, is reactive Langerhans cell hyperplasia

Eosinophils, hallmark of eczema is also present in LCH and infectious diseases (prominent in parasitosis, in mycosis but also in some cases of HSV lesions!). Eosinophilic spongiosis: think Pemphigus or Incontinentia Pigmenti.

Neutrophils (epidermis / stratum corneum): psoriasis, impetigo, mycosis but also sometimes seen in Zoon's vulvitis.

Plama cells: mild infiltrate of plasma cells is nearly always present in mucosal biopsies. It is a hallmark of Zoon but in context of psoriasiform or/and lichenoid pattern (with/without granuloma) syphilis must be excluded.

Melanocytic tumors

Introduction
As in other locations, to reach a correct diagnosis of melanocytic lesions clinical data are crucial: age of the patient (no age = no diagnosis!), ABCDE criteria (Asymmetry, Border, Color, Diameter,Evolving), dermatoscopic features and precise location of the genital lesion. Indeed melanoma are more frequent on glabrous skin and mucosa whereas atypical melanocytic nevi of genital type (AMNGT) are usually located on non-glabrous skin.
Pigmented Macules / Genital melanosis
is defined by melanin pigmentation of basal melanocytes and keratinocytes with no melanocytic proliferation.

Common Nevi / Lentigo
Most genital nevi or lentigo do not have peculiar histological characteristics. "Banal" junctional, with or without bland dermal component are observed.

Atypical melanocytic nevi of genital type
AMNGT is challenging and account for 5-25% of genital nevi. They occur in children, teenagers and young females (29y, range 8-42) and the lesions are small (around 0,7cm). Histologically, they are sharply circumscribed and characterised by intense and disorganised junctional melanocytic proliferation characterised by nests variable in shape and size and areas of confluence. Nests are composed of large / epithelioid discohesive atypical melanocytes. Pagetoid spread can occur usually restricted to the centre of the lesion. Lentiginous proliferation pattern is only mild (in contrast with genital melanoma where lentiginous pattern dominates). If intradermal component exhibits atypia, they are confined to the superficial dermis. Sparse non atypical mitosis can be observed. AMNGT have frequent Braf mutation in contrast with genital melanomas that show KIT mutation and many copy number aberrations. AMNGT must be recognised to avoid useless mutilations in young patients! It must be noted that punch/incomplete biopsy is must be avoid (cf impossible evaluation size, symmetry, lateral demarcation…). AMNGT can be included into the group of nevi at special sites (ie: nevi of axilla/folds, umbilicus, ear…). These nevi have features simulating melanoma but "atypical" term is only linked to their peculiar histological aspect. These nevi are benign and no tendency to evolve into melanoma.

Blue Nevi and Spitz nevi
Rarely been reported in the vulva.

Melanoma
Genital melanoma occur in older patients than AMNGT (66y, range40-94) and are larger (around 2 cm). The tumour is asymmetric, poorly demarcated and usually characterised by disorganised lentiginous and confluent junctional proliferation of highly atypical melanocytes with hyperchromatic irregular nucleielongated and vertically oriented. Junctional melanocytic proliferation often involve adnexal structures. Dermal component could be desmoplastic and neurotropic. Kit mutation is present in15-40% of mucosal melanoma Nras mutations in 15% of the cases. Braf mutation is usually absent and multiple genes amplification is also an usual features. Typical SSM can also be observed.

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INVITED LECTURES

L 2

VULVAR PATHOLOGY - SOFT TISSUE TUMORS.

Nicolas de Saint Aubain
Institut Jules Bordet, Brussels

A wide variety of soft tissue tumors may arise in the vulvar/perineal soft tissue (schwannoma, leiomyoma…), but a few entities arise specifically in that area: cellular angiofibroma, deep angiomyxoma, angiomyofibroblastoma, proximal-type epithelioid sarcoma.

Fibroepithelial polyps are also common in that location; they may exhibit worrisome atypical features (nuclear pleomorphism, mitotic activity) and are often confused with deep angiomyxoma.

CALME is a very uncommon hyperplasia of the vulvar stroma affecting children.

Cellular angiofibroma (1-4)

Cellular angiofibroma belongs, with spindle cell lipoma and mammary-type myofibroblastoma, to a group of tumors characterized by deletions of 13q (including RB1 gene).

Males and females are equally affected, with a peak of incidence in the 5th-7th decades.

Tumors are well-circumbscribed and resemble spindle cell lipoma: they are composed of bland spindle cell with oval nuclei and bipolar cytoplasmic expansions. Small aggregates of adipocytes are present in up to 50% of cases. Most tumors stain for CD34. Immunostaining for RB1 could be useful but is often difficult to interpret.

Recurrences are uncommon. Rare cases with malignant transformation have been reported.

Deep ("aggressive"') angiomyxoma (5,6)

Deep angiomyxoma present as a small growing, poorly circumscribed mass, in the pelvicoperineal soft tissues of adult females (30-50 yo).

Tumors are often large, composed of spindled and stellate cells embedded in a myxoiod matric containing hyalinized vessels. Scattered myoid cells with eosinophilic cytoplasm are often identified around vessels.

Most cases express HMGA2. Desmin and actin staining is variable.

Although deep angiomyxoma may recur in up to 35% of cases; recurrences are usually not destructive and can be controlled by simple reexcision. A few recent studies had demonstrated excellent local control with conservative surgery.

Angiomyofibroblastoma (7)

Angiomyofibroblastoma present as a well-circumscribed nodule in the pelvic/perineal region, especially the vulva. Most cases affect adult females; rare cases occur in males.

Tumors are well-circumscibed, composed of round to spindle-shaped eosinophilic cells, which tend to concentrate in clusters around vessels. The stroma is loose, edematous. The vascularization is prominent; mostly composed of thin-walled vessels. Rare case with hyalinized vessel walls show some overlap with deep angiomyxoma.

Most cases stain for desmin and smooth muscle actin

Recurrences are exceedingly uncommon.

Fibroepithelial polyp (8)

These lesions occur at a wide age range. When typical, they are easily recognized. However, they may occasionally display worrisome pseudosarcomatous features: hypercellularity, nuclear pleomorphism, high mitotic activity or the presence of abnormal mitoses. Stromal cells variably stain for smooth muscle actin and desmin (but myogenin is negative, allowing the distinction from botryoid rhabdomyosarcoma).

Practical approach:

- Polypoid lesion: Fibroepithelial polyp
- Poorly circumscribed mass: Deep angiomyxoma
- Well-circumscribed mass:
    - o CD34+, Rb1 -, spindle-cell lipoma-like: cellular angiofibroma
    - o Actin +, Desmin +/-, clusters of eosinophilic cells: angiomyofibroblastoma

CALME (Childhood Asymmetric Labium Majus Enlargement) (9)

CALME is a very uncommon lesion characterized by enlargement of one or occasionally both labia majora, which affects young girls (4-13 yo). The histological appearance is not specific as the lesion if formed of usual components of the vulvar stroma: lobules of fat are encircled by moderately cellular fibrous septa.

Recurrences may occur.

CALME is regarded as a physiologic enlargement in response to hormonal stimulation rather than as a neoplasm.

Proximal-type epithelioid sarcoma (10)

Proximal-type epithelioid sarcoma (ES) is an uncommon variant of epithelioid sarcoma that tends to affect the pelvic/perineal area and proximal limbs.

The characteristic granuloma-like appearance of typical ES tends to be replaced by a sheet-like growth pattern. Tumor cells are large, with a prominent epithelioid or rhabdoid appearance.

All cases of proximal-type ES stains for cytokeratins and EMA, as well as CD34 in more than 50% of cases. Loss of IN1-1/SMARCB1 is characteristic and allows the distinction from poorly differentiated carcinoma.

Prognosis is poor (distant metastases to lymph nodes, lungs, in 40% of cases).

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INVITED LECTURES

L 3

EPITHELIAL TUMOURS OF THE VULVA.

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Most of the vulvar tumors are from epithelial origin, and are generally from glandular or squamous origin. These tumors are either benign or malignant and mau be for some of them preceded by epithelial precursors in squamous lesions.

From them squamous lesions and their precursors called vulvar intraepithelial neoplasia/VIN are the more frequent.

Classically, concerning the epithelial precursors, are observed:

1) Low-grade squamous intraepithelial lesion/VIN1 are for the majority of them associated with HPV infection low and/or high risk and there is an overlap for some of them with condyloma with different p16 expression. These lesions usually regress.

2) High-grade squamous intraepithelial lesiosn (VIN2/3) are also HR-HPV associated with or without cervical and/or anal coexisting infection. Generally, in these lesions a diffuse p16 expression was noted. The progression to invasive carcinoma is rare.

3) Vulvar differentiated-type intraepithelial neoplsia are classically HPV negative and is frequently associated with chonic dermatosis. The diagnosis of this entity is subtil and differentail diagnosis with the so-called squamous hyperplasia remains difficult. A mutation of p53 can be observed with consecutive p53 protein expression and the risk of progression to invasion more frequent than with VIN2/3 HR-HPV induced lesions.

Invasive squamous cell occurs generally in older women at least for those not HPV-associated. Different variants are observed including warty, keratinizing, verrucous types,… The prognosis of these tumors is intimately related the the WHO stage and the multifocality of the lesions.

Glandular lesions at least those with malignant potential are quite rare and described in detail in the oral presentation.

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L 5

DYSFUNCTIONAL PROLIFERATIVE AND SECRETORY ENDOMETRIAL DISORDERS AND DYSFUNCTIONAL UTERINE BLEEDINGS.

Claire Bourgain, MD, PhD

Introduction

When evaluating an endometrial biopsy for any purpose, an adequate clinical history is important, including the age of the patient and the reason for biopsy. According to the patient's age, endometrial anomalies occur at a different frequency. The menopausal status as well as intake of any exogenous hormonal therapy should be recorded. In ovulatory patients, ideally, the day of ovulatory-inducing LH- surge should be monitored. As this is rarely performed outside a fertility treatment, at least the day of onset of latest menstruation and average length of the menstrual cycle should be provided. (1)

Definitions

Dysfunctional maturation is a disparity between the clinical menstrual cycle day and the endometrial microscopic changes

Most conditions of dysfunctional maturation will eventually cause endometrial bleeding: According to the recent PALM-COEIN FIGO classification (2), the term *dysfunctional endometrial bleeding* should be discarded. Disturbances of regularity, frequency, heaviness or flow in bleeding can be caused either by structural changes (Polyps, Adenomyosis, Leiomyoma and Malignancy) or non-structural causes (Coagulopathy, Ovulatory dysfunction, Endometrial causes, Iatrogenic causes and Not yet classified causes)

Underlying mechanisms of abnormal endometrial bleeding

Abnormal endometrial bleeding (AUB) is a common problem in women in the 30-50 age group. It is defined as excessively heavy, prolonged or frequent bleeding of endometrial origin, which is unrelated to pregnancy or a recognizable pelvic disease. Most causes are related to anovulatory cycles or luteal phase anomalies. Especially in the perimenopauzal phase, an endometrial biopsy should be performed to rule out hyperplasia or malignancy.

General mechanisms of bleeding are still incompletely understood, but due principally to impaired hemostasis, increased angiogenesis or both. Bleeding usually reflect the absence of the hemostatic effect of progesterone on TF (tissue factor), HESC (hemostatic factor) and its inhibitory effect on MMP (matrix metalloproteinase) 1, 3 and 9 to stabilize the stromal and vascular extracellular matrix. Unrestrained angiogenesis is observed with long-term progestin only contraception, myomas and polyps (3)

Evaluation of endometrial biopsies in AUC and related disorders

Endometrial biopsy should be carried out as a first diagnostic step if imagery reveals no pelvic anomaly and after exclusion of bleeding dyscrasias especially Von Willebrand's disease (responsible for 5-20% of AUB).

Adequacy and artefacts should be evaluated before endometrial tissue can be assessed. At present, there are no standardized criteria upon what is an adequate biopsy. Tissue from the cervix and myometrium may often be present. As a general rule, biopsies containing no endometrial tissue should be regarded as inadequate. Biopsies permitting a histopathological diagnosis, albeit containing very scanty tissue should be regarded adequate. Small endometrial tissue fragments not permitting a definite diagnosis should better be quoted not assessable than inadequate. Artefacts derived from biopsy telescoping or compression should be recognized an non-pathological conditions. Presence of lower uterine segment and isthmus tissue should not be confused with an endometrial polyp.

1. Proliferative and secretory disorders in ovulatory patients

Inadequate proliferative phase

This condition is characterized by a proliferative endometrium, but there is a discrepancy between the clinical cycle phase and the endometrial maturation. Usually the endometrium will be delayed with respect to the clinical cycle day.

Proliferative endometrium should always contain mitosis, although they may be small in number. A proliferative endometrium without other anomaly is

the most frequent endometrial pattern observed in patients with abnormal endometrial bleeding.

Luteal phase defect and abnormal secretory patterns

It should be kept in mind that luteal phase defect is a clinical and not a pathological term, and its significance as a cause of infertility is greatly debated. This condition has been implicated in abnormal uterine bleeding. Its underlying mechanism is multifactorial and still not completely understood.

The best method is to recognize when secretory features are inconsistent to a certain pattern of 'datable' changes. *Inadequate secretory endometrium* is characterized by a difference of >2 days with the clinical cycle day in at least two consecutive biopsies. The endometrium shows underdeveloped secretion with the persistence of thin, uncoiled glands with minimal vacuoles and thin arterioles. Bleeding occurs if there is an inadequate amount of progesterone to sustain the normal secretory development. Focal breakdown with 'blue balls' and karryorrhectic debris are observed in an otherwise non-menstrual secretory endometrium.

More rarely, there may be a more localized alteration affecting the glands only. This phenomenon *is quoted irregular endometrial ripening* or *mixed secretory pattern* and is characterized by an admixture of secretory and foci of non-secretory epithelial changes. Completely *dysynchronous* or *asynchronous endometrial-stromal maturation* can also be observed and is a frequent pattern in ovulation induction therapy (5). Localized phenomenon of irregular ripening points to a primary endometrial anormality rather than a central endocrinological or corpus luteum defect. Asynchronous endometrial glands (AEG) show either a proliferative or inactive non-secretory phenotype. They correspond to a primary gene- inactivating event within the progesterone response cascade and thus have an alterated progesterone response. Complete loss of PTEN and PAX2 function due to primary genetic defects will lead to a completely negative AEG's for those markers. In case of a failure in the progesterone receptor cascade, persistent estrogen receptor alpha and beta and progesterone receptor as wel as increased PAX2 and Ki67 expression in contrast to the adjacent secretory glands will be observed (6, 7). When abnormal secretory patterns are the result of organic anomalies such as inflammation, myoma, polyps, abortion or ectopic pregnancy, the primary pathology may not be present in the biopsy. In those cases, it is not possible to determine the underlying causes, consequently a descriptive diagnosis should be assigned.

Irregular shedding and membranous dysmenorrhea

Irregular shedding is an unusual event attributed to a persistent corpus luteum with prolonged progesterone production. It is composed of an admixture of secretory and proliferative glands at least 5 days after onset of bleeding. There are foci showing more than 4 days difference in the morphological date. Breakdown and bleeding with stromal collapse are present.

Dysmenorrhea membranacea (synonym decidual cast) is a clinical term involving spontaneous shedding of the endometrium in one or several membranous pieces retaining the shape of the uterine cavity. This rare condition is seen mainly in young women between 20 and 40 years. It is considered as a variety of irregular shedding of the endometrium. There are several hypothesis concerning its etiology, being raised production of estrogen and progesterone with partial dissolution of thickened endometrium. Another theory suggested a normal but intense development of spiral arteries with excess vasodilatation and then intense vasoconstriction with shedding of an overdeveloped endometrium. or hyperprogesteronism which can be caused exogenously or endogenously . Histologically, decidualized endometrium with regressive changes or focal hemorrhagic necrosis and infiltrated by neutrophils is observed. The decidual cells may have a spindly shape when regression is advanced. The endometrial glands are lined by cuboidal epithelium. Ectopic pregnancy should always been excluded.

2. Disorders associated with anovulation

Proliferative endometrium with breakdown is the most frequent cause for bleeding in the perimenopausal woman or women with polycystic ovary syndrome. The mechanism is unopposed estrogen effect. The microscopic pattern will vary according to the duration of unopposed estrogen. Sporadic anovulation will lead to small glands with weak proliferation. In long-term unopposed estrogen, the condition may evolve to *disordered or persistent proliferation*. Microscopically, there is focal cystic dilation of glands that do not have (glandular) secretions. Glands are >2x larger than their normal size, usually 3-4x normal. The glands have an irregular shape, e.g. gland contour has inflection

points. More than four glands should be involved (dilated). There is variable stromal condensation due to breakdown of endometrium). Dilated glands often have tubal metaplasia and small vacuoles, which are less important than those observed in early secretory endometrium. The condition is associated with eosinophilic syncytial metaplasia and may evolve to or be associated with hyperplasia.

Atrophy is an important cause of bleeding in postmenopausal patients. Usually, scant tissue is obtained. The epithelium is columnar with minimal cytoplasm. There may be cystic changes. The stroma consist of dense spindle cells. There is no mitotic activity.

3. Exogenous hormonal effects on the endometrium (8)

Exogenous steroid applied as female contraceptives

The classical combination oral contraceptives contain both exogenous estrogen and progestins. After a few cycles, the progestin will down-regulate the estrogen receptor leading to small atrophic glands and an atrophic or variably decidualized stroma. There is a decrease in vascular density and dilated superficial microvessels. This image is *quoted arrested secretion or pill endometrium*.

In progestin-only oral preparations, a proliferative or deficient secretory pattern can be observed. Atrophy may be seen in long-term users, but the stroma is more abundant as compared to normal atrophy.

Subcutaneous implants of progesterone lead to a variable non-specific range of endometrial changes, from atrophy to normal endometrium or progestin effect. Injectable progesterone will initially induce more marked progesterone effects with Arias-Stella reaction and decidua, but will eventually lead to atrophy. Intra-uterine devices with progesterone will lead to a superficial decidual reaction of the stroma and atrophic glands.

Selective estrogen receptor modulators (SERM)

Tamoxifen used as hormonal therapy for breast cancer is the most used and probably best studied SERM. It acts both as an estrogen agonist and antagonist. Most frequently, it induces polyps with various changes in the glands and stroma. Not infrequently, hyperplasia and carcinoma may arise after tamoxifen therapy (9)

Selective progesterone receptor modulators (SPRM)

SPRM are used in contraception, management of fibroids and endometriosis and as an abortion inducing drug. They bind to the progesterone receptor and induce specific agonist, antagonist or mixed activity. The endometrial changes induced by SPRM (PAEC) are specific to these type of molecules and have to be distinguished from true hyperplasia. The drugs cause cystically dilated glands with only weak mitotic activity and occasional secretory vacuoles pointing to a non-physiological progesterone effect. Other findings are thick-walled vessels and anastomosing capillaries (10).

4. Endometritis

In case of AUB and/or undatable endometrium, chronic endometritis is one of the possible, although less frequent pathologies. The presence of plasma cells can be highlighted by CD138 immunostaining.

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INVITED LECTURES

L 6

EVALUATION OF POTENTIAL MEDICAL MISTAKES BY PATHOLOGISTS: THE BELGIAN PROCEDURE.

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Medical mistakes by pathologists seldom lead to trials in Belgium. Information obtained from a major belgian insurance company for medical doctors indicates that the insurance was involved in only 8 cases since 2009, of which only 5 were referred to a lawyer in preparation for defence at trial. Although not comprehensive for all cases of pathological mistakes with patient complaint in Belgium, this number is negligible compared to all cases of medical mistakes managed by this insurance company. Nevertheless, the number is increasing in the last 5 years since no case was recorded before 2009.

One of these mistakes was a wrong diagnosis of benign melanocytic naevus, compound type, that appeared to be a malignant melanoma when metastases occurred in draining lymph nodes almost 5 years later. Despite treatment, including several surgical procedures and inclusion in novel personalized therapeutic trials, the disease progressed and the patient died of disseminated melanoma 12 years after excision of the primary lesion.

All 4 experts who revised the initial lesion concluded to a mistake, but of course the diagnosis was more obvious with knowledge of the clinical evolution. Actually, the lesion was a largely excised small superficial spreading malignant melanoma, with no ulceration, focally infiltrating papillary dermis to less than 0.3 mm in depth. Prognosis of such a limited melanoma, Clark level 2, T1 stage, is thought to be good with less than 2% risk of lymph node metastasis (Breslow, Ann Surg 1975, 182: 572-5). Therefore, the awesome evolution of the lesion could not be predicted, and one wonders whether development of lymph node metastasis almost 5 years after excision of the primitive lesion could have been prevented, even if the correct diagnosis had been made initially.

This example of wrong diagnosis highlights the distinction that has to be made between a medical mistake and a fault. Belgian jurisprudence consistently indicates that a misdiagnosis is not a fault by itself, but that demonstration must be made that all available means were not used to reach the correct diagnosis. One has to examine whether a normally cautious and qualified medical doctor could also make a wrong diagnosis in the same case and in the same circumstances (translated from Génicot, Droit médical et biomédical, 2010, Larcier, p. 371). One unfortunate side of the presented example is that the dermatologist expressed her concern to the pathologist regarding the initial diagnosis of benign lesion that did not fit with the clinical appearance of the lesion, but the pathologist did not reevaluate the diagnosis nor ask for a second opinion, which would most likely have prevented patient complaint whatever the second opinion diagnosis would have been. The case will now be brought to the court and one will see how it will be judged.

The pathologist can also be involved in trials of medical cases through his report, even without knowing it. As an example, the pathological report of an hysterectomy specimen has been used by a lawyer in a trial against an obstetrician, to contest the clinical diagnosis of disseminated intravascular coagulation that justified hysterectomy for massive post-partum metrorrhagia because no evidence of intravascular thrombus was found on the histological sections.

Finally, expertise of the pathologist may also be requested to clarify some allegations in criminal cases as will be illustrated in a case judged at the Winchester Royal Court. The accused man pretended the blood stain on a map found in the hut where he was hiding but finally arrested was menstrual blood of the murdered woman with whom he had sexual intercourse before she was slaughtered by some unknown thief. Molecular analysis of the blood stain was performed to intend to demonstrate whether it was due to menstrual or to peripheral blood.

In conclusion, only those who make no diagnosis will do no mistake. But it is expected from the pathologist that he helps clinicians to reach the most accurate diagnosis as possible, while remaining cautious when all contributive information is not available or concordant. The pathologist has no contact with the patient but he may interact with the clinician who is in charge of the patient and he should take into consideration any discordant clinical information he gets, and possibly ask for a second opinion if needed, before reaching a final diagnosis.

L 7

THE BELGIAN ORDER OF PHYSICIANS: ADVISING, SUPPORTING, SANCTIONING.

Dr Louis THIENPONT, Member of the Belgian Order of Physicians, Provincial Council of East-Flanders

The Order of Physicians, a public-law institution with legal personality, was founded by the law of July 25th, 1938, but because of the Second World War, this law only entered into force on June 13th, 1947. In 1967 a few amendments to the law were introduced by the Royal Decree of November 10th, 1967. In order to be gender neutral, the Dutch name of the Order of Physicians was changed from 'Orde der geneesheren' to 'Orde der artsen' on January 19th, 2016. The President and Vice-President of the National Council are entitled to validly represent the National Council in and out of court. Each physician has to be registered on the List of the Provincial Council where he/she has his/her main professional activity. The Order of Physicians is funded by the annual dues of the members.

The **Order of Physicians** consists of the National Council, ten Provincial Councils and two Councils of Appeal (Dutch and French).

Further items to be discussed are the 'Code of Medical Ethics', 'Physician in Difficulty', and some projects to reform the Order of Physicians.

The National Council

The National Council has two sections: one Dutch-speaking and the other French-speaking. Each section counts:

- 10 members delegated by the Provincial Council for six years,
- 6 members designated by the King for six years upon recommendation of the medical faculties,
- 1 registrar designated by the King for six years,
- 1 President designated by the King among the (honorary) judges of appeal of the Court of Cassation.

For each above-mentioned position, a substitute is elected or designated. Finally, the Dutch and French-speaking sections each elect a Vice-President who is also vice-president of the National Council.

Assignments of the National Council

1. To determine the Code of Medical Ethics
2. To keep a register of disciplinary decisions that are no longer subject to appeal
3. To give a motivated reply to deontological issues
4. To fix the annual dues for physicians (and the contribution to the National Council)
5. To issue legal certificates for physicians who want to practise in other EU Member States
6. To communicate regarding disciplinary sanctions in relation to practice of medicine in other EU Member States

Commissions of the National Council

These are, among others:

- Medical Professional Companies, Partnerships and Contracts
- Code of Medical Ethics
- Hospital Medicine
- General Practitioners
- Reform of the Order of Physicians
- International matters
- Etc.

Provincial Council

The number of members for each Provincial Council is determined by the King. Members (f. ex. for East-Flanders: 14) and substitute members are elected for a term of six years.

The conditions for eligibility of members are:

- to have the Belgian nationality,
- to be registered on the List of one of the Provincial Councils for at least ten years,
- to be registered on the List of your current Provincial Council for at least one year, and
- to have received no other disciplinary measures than a 'Warning'.

Voting is mandatory.

The two advisory members are a councillor of a court of first appeal appointed by the King and a member (or substitute member) of the National Council for a six-year term.

INVITED LECTURES

Authority of the Provincial Council

1. To establish the list of the members of the Provincial Council
2. To give advice in matters of medical deontology
3. To guarantee compliance with the Code of Medical Ethics: disciplinary jurisdiction over deontological faults in medical practice or related situations involved
4. To inform the qualified authorities of cases of illegal medical practice
5. To decide as a last resort on disputes between physicians and patients over medical fees
6. To advise courts of justice about disputes regarding medical fees
7. To fix the annual dues

Activities of the Provincial Council

(Mentioned here are the activities for 2014 of the Provincial Council of East-Flanders.)

1. Council meetings (20 meetings)
2. Meetings of the Board (President, Vice-President, Secretary, Legal Councillor, Member National Council) (26 meetings, 769 records handled)
3. Two investigation committees (40 meetings, 70 physicians heard)
4. Committees for Contracts and Medical Practice (21 meetings, 362 records handled)
5. Committee Company Statutes and Partnerships (23 meetings, 425 records handled)
6. Financial supervision (scrutatores)
7. Websites

Disciplinary jurisdiction

Investigation of complaints in the Provincial Council of the Order of Physicians:

1. Board: Every complaint sent by letter or e-mail is taken into consideration. The Board can mediate between parties or else refers the complaint to the investigation committee.
2. Investigation committee: composed of substitute members of the Provincial Council appointed by the Board. This committee always meets in the presence of a councillor who guaranties the procedure, the objectivity of investigation and the respect for the rights of all parties involved. The investigation committee examines inculpatory and exculpatory evidence. It is the commission's duty to find out the truth. The commission can invite both accused and complaining parties. If necessary, witnesses and experts can be summoned.

Investigation procedure

The physician receives a copy of the complaint and information about the exact facts and circumstances as well as the identity of the complainant. The physician is first invited to give his own account of the facts in writing.

Next, either the physician is invited by the committee in order to give an oral explanation, or else the case is sent directly (without interrogation) to the Provincial Council for assessment. The Council can then dismiss the case or request that the physician be summoned before the committee. The physician can be assisted by a counsel (Saldus) who may not intervene at that moment and is only allowed to care for the rights of the defendant.

After the interview with the investigation committee, a report is sent to the physician, who can add comments (his remarks) within 15 days of receipt. The investigation committee then makes its final report to the plenary session of the Provincial Council.

The Council takes note of the report and decides that either the case should be dismissed because of lack of incriminating facts, or that additional investigations are required.

If needed, the charged physician then is further interrogated by the committee, or additional witnesses are heard, or there can even be an additional investigation. If not, the physician must appear before the Council.

Appearance before the Council

The physician concerned is invited by registered letter no later than 15 days before the meeting. The invitation mentions the complete accusation in detail. The accused physician and possibly his counsel have access to complete information and can obtain copies of documents if wanted.

After interrogation and defence, the charged person leaves the Council meeting. The Council deliberates and decides if the physician has really committed a deontological fault or not. The sentence is addressed to the accused by registered letter within 8 days.

Sanctions as defined by law (Royal Decree of February 6th, 1970)

- *Moral sanctions:* warning, censure, reprimand
- *Suspension:* 1 day up to 2 years*
- *Erasure* from the List*
- *Acquittal* if accusations are not proven or if there are strong extenuating circumstances and/or evident grounds for clearing or excuses

*For a suspension of more than 1 year and for erasure from the List, a two-thirds majority of votes is required.

Note

All sessions of the investigation committee at a provincial level take place "in camera". The law does not allow the sentence to be communicated to the complainant (change in the future?). Every member of the Council is bound by professional secrecy.

Council of Appeal

For both linguistic systems, five members are appointed by each Provincial Council for six years and five members who are councillors of the Court of Appeal are appointed by the King for six years. For each of these positions (2x5), there are substitutes. Finally, one delegate of the National Council is also a member of the Council of Appeal.

Competences of the Council of Appeal

- Deciding on appeals from decisions pronounced by the Provincial Councils
- Entitled to judge in first and final instance in case of objections against results of elections and in matters of expiration of the mandate of a member of the Provincial or National Council or the Council of Appeal, who was given a deontological sanction against which appeal is no longer possible, or who was sentenced for criminal facts that prove that this person is morally or professionally unworthy to continue functioning in his mandate.
- Entitled to judge in case of a dispute between Provincial Councils about the medical domicile of a physician, being the address of his main professional activity.

Code of Medical Ethics

The Code of Medical Ethics constitutes the set of rules, behaviour and common practice that every physician must respect or must follow in his/her daily practice. The code counts 182 articles.

1. General rules (quality of care/publicity/clients)
2. Physician's service towards patients (quality of care/medical records/professional secrecy/end of life – euthanasia)
3. Physician in service of the community (social and economic responsibility of the physician/ continuity of care/physician on duty and emergency service)
4. Relations between physicians (collegiality and professional collaboration)
5. Relations between physicians and third parties (contracts with welfare and nursing institutions/ relations with pharmacists, dentists and paramedics)

The code dates back to 1973 and has not been ratified by a ministerial decree. The **Court of Cassation** decided, however, that the lack of ratification does not diminish the binding force of the rules of the Deontological Code.

The **National Council** puts forward that the rules of the Code must be considered to be a piece of compulsory advice, with imperative advisory power for physicians. Therefore, the rules of the Code of Medical Ethics are pertinent cornerstones for disciplinary proceedings by the Provincial Councils and Councils of Appeal. If necessary, the National Council can adapt the terms of the Code to comply with the current medical practice.

Each year around 400 complaints are addressed to the ten Provincial Councils of Belgium. Around 50% of these complaints are dismissed. Of the remaining half, around 25% get acquitted and around 75% are sanctioned with a moral sanction, suspension or even erasure from the List.

Table 1. Number of disciplinary sanctions in Belgium 2005-2015

DISCIPLINARY DECISIONS FROM 01-01-2005 UNTIL 31-12-2015 PER YEAR									
YEAR	TOTAL/YEAR	REPRIMAND	CENSURE	WARNING	SUSPENSION	ERASURE	ACQUITTAL	PATERNAL EXHORTATION	WITHOUT CONSEQUENCES
2005	369	30	9	46	62	4	29	0	189
2006	343	28	7	37	59	3	42	0	167
2007	346	32	6	47	63	4	47	2	145
2008	394	27	5	28	75	5	48	0	206
2009	415	45	4	40	62	2	29	0	233
2010	274	22	5	24	50	2	36	0	135
2011	450	35	8	29	63	3	64	0	248
2012	412	26	4	41	56	2	47	0	236
2013	340	25	1	35	54	6	51	0	168
2014	427	23	4	31	41	7	45	1	275
2015	414	32	4	24	42	5	46	0	261
TOTALS	4184	325	57	382	627	43	484	3	2263

Table 2. Disciplinary sanctions in Belgium 2005 -2015 (suspensions detailed)

DISCIPLINARY DECISIONS FROM 01-01-2005 UNTIL 31-12-2015 SUSPENSIONS DETAILED PER YEAR									
Year	1-7 days	8-15 days	1 month	2 months	3 months	6 months	1 year	2 years	TOTALS
2005	21	9	12	3	6	4	4	0	59
2006	31	8	9	3	5	4	1	1	62
2007	21	15	11	6	3	2	4	0	62
2008	22	15	12	9	3	6	8	1	76
2009	21	11	4	6	3	4	9	2	60
2010	22	3	13	3	2	3	2	1	49
2011	30	13	11	3	3	1	1	2	62
2012	28	8	9	1	4	7	0	1	59
2013	21	9	10	3	3	4	3	0	53
2014	13	5	2	4	9	3	4	1	41
2015	16	5	10	6	1	3	2	1	44
TOTALS	246	101	103	47	42	41	38	9	627

Is a reform of the Order of Physicians imminent?

In the years 2014-2015, experts and politicians exchanged opinions. In June 2015, a concept paper of reform was submitted to the Ministry of Health.

Situation in September 2015: proposals for reforming the **position of the complainant**, relation mediation/ombuds service, relation Order of Physicians/provincial medical committee, interdisciplinary and multidisciplinary collaboration, guarantees for quality of care, patient care, community interest and care, positive deontology and increase of transparency, quality of disciplinary jurisdiction, new procedures and structures.

Situation today, in September 2016: change of name 'Orde der artsen'; electronic elections instead of paper voting, administrative district instead of judicial district for candidates and elections.

'Physician in Difficulty'

Physicians must help each other (art. 11 of the Code). Recently, the project 'Physician in Difficulty' (Arts in Nood/Médecins en Difficulté) was created in order to help physicians with personal problems. 'Physician in Difficulty' operates as an independent entity that is, however, financially supported by the Order of Physicians. The project was initiated in 2013 by the Provincial Council of East-Flanders by Dr Michel Bafort.

'Physician in Difficulty' is a national contact point for:

- physicians in psychological suffering, requiring contact with a person in whom they can confide,
- personal problems, burn-out, alcoholism that result in inadequate medical functioning, or
- contact in case of aggression.

As of August 1st, 2016, one single national phone number, **0800 23460**, has been established. It provides permanence by a multilingual person, who guides the physician in need to a network of persons of confidence. The operational expenses are funded by the Order of Physicians, who is **otherwise uninvolved**. All persons involved are bound by professional secrecy. The 'Physician in Difficulty' can renounce at any moment; his agreement must be obtained for every step in the guiding procedure.

The official nationwide launch is planned on October 25th, 2016, during an academic session in the Club of University Foundations in Brussels.

Conclusion

In the last decade, the Order of Physicians (Orde der artsen – Ordre des médecins) has, rather slowly, made efforts on the way towards reform and modernisation. Every step needs political and social approval and an amendment of the law. Efforts to change into a more supportive and advising institution, rather than a punishing one, have shown positive results. Nowadays, the Order of Physicians absolutely prefers to advise, inform physicians and assist them in case of problems. It intervenes and mediates whenever conflicts arise. The project 'Physician in Difficulty' has had a promising start.

Considering the receipt of around 400 complaints per year, disciplinary jurisdiction over deontological faults remains an important task. This is in fact a jurisdiction by peers, elected members of the Provincial Council of the Order of Physicians. The high number of complaints per year serve as proof that the role of the Order of Physicians is recognized in our society.

For all these reasons, and after seven years of inside experience, I am convinced that if the Order of Physicians had not yet been in existence, we would now have been in urgent need to found it.

I wish to thank Dr Piet Van Mulders, Member of the National Council, and Mrs Marina Dillen, Administrative Director of the National Council, for their contribution to this article.

INVITED LECTURES

L 10

PROCEDURES IN CASE OF MEDICAL MISTAKES BY PATHOLOGISTS. EXPERIENCES FROM THE NETHERLANDS

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Suspecting and determining an ex-post erroneous pathologic diagnosis is a nightmare for both the patient as well as the pathologist, especially if this has significant consequences for the patient.

What should we as pathologists do, and what can we expect?

First of all, the applicable local procedures for reporting must be followed, and in consultation with the requesting specialist of the examination in question and the complaints officer of the hospital, it should be discussed who informs the patient. The pathologist should be prepared to fulfil an understanding and informing role.

Past experiences show that the quality of these first dialogues can be decisive for the later stages of the process.

Does the patient file a formal complaint against the pathologist? Is the pathologist held responsible for the alleged damage and is a claim filed? Is the pathologist's practise experienced as severely insufficient and is this reported to the Health Care Inspectorate or the Medical Disciplinary Council?

In that event, it has to be established whether the pathologist has acted according to the professional standard and, as the Supreme Court of the Netherlands has determined: "with the care that may be expected of a reasonably practising and reasonably competent professional in equal circumstances".

Since this *standard* can basically only be determined by professional colleagues (naturally, not the legal judgement regarding culpability), an experts investigation will normally be requested.

The Dutch Society of Pathology has developed a protocol for this (first published in the NTvG, 2000; 144; 566, Giard and Broekman).

The "expert" is a pathologist with experience in the area of experts investigations and not necessarily an expert on the relevant field of pathology. This expert systematically analyses the possibly ex-post erroneous diagnosis, which includes the following elements: the scientific context of the contested examination at the time of the primary assessment, the clinical context, process testing and mostly a revaluation of the contested pathological examination.

This revaluation will be organised by the expert and consists of putting together a set of 5 similar, completely anonymous pathological examinations including the contested examination, which will be submitted for revaluation to 5 pathologists, independent of each other and without knowledge of the cause or outcome of the case, with the literal clinical information that was available for the primary assessment.

The expert reports the analysis as described above and the findings/results of the revaluation and the interpretation thereof.

This procedure will be clarified in the presentation by using a few examples.

L 12

WHAT IS NEW AND RELEVANT IN EPIDERMAL TUMORS.

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Few areas of Dermatopathology were thought to have such a steady basis as the field of epidermal tumors. Yet, the introduction of breakthrough technologies leading to new scientific achievements, on one hand, and the erosion of well-established concepts by confuse ideas, on the other, make necessary to reconsider and periodically update this area of skin pathology.

A milestone in the study of epidermal tumors was the finding of a new polyomavirus (MCV) clonally integrated in 70 to 80% cases of Merkel cell carcinoma (MCC) that contributed to explain why this tumor not only occurs on sun-exposed skin areas from elderly patients, but also in patients with organ transplantation, chronic lymphatic leukemia and AIDS.(1) Lately, MCV has been found in other cutaneous and non-cutaneous processes. (2) A pathogenic role in all of them has not been stablished with feasibility, yet. At any rate, the viral origin of many MCC cases has led to divide this neoplasm into two definite variants, (3) (4) with different prognosis and even the term neuroendocrine tumor of the skin has been suggested for the non-viral cases. Besides, the existence of an infectious trigger seems to enhance the immune response. This can explain the cases of MCC with spontaneous regression and has been the germ for the successful idea of treating this aggressive neoplasia with immunomodulatory therapies. (5)

Under the microscope, one of the main differential diagnosis of MCC is basal cell carcinoma (BCC). The similarity between these two neoplasms is strengthened by the expression of neuroendocrine markers in some cases of BCC. It has recently been remonstrated that this neuroendocrine differentiation is associated to resistance to Vismodegib, an effective targeted therapy for many advanced BCCs. (6) The introduction of these new treatment has been a stimulus to report numerous cases of metastasizing BCC. The existence of life threatening cases had been probably silenced for years to avoid being judged as a diagnostic error. Conversely, the existence of locally aggressive BCC variants, such as micronodular and basoescamous BCCs was well known. A new classification for high risk BCC variants has been recently proposed with suggestions for the pathology report. (7)

Regarding squamous cell carcinoma, in the last years the interest has been focused in the intraepidermal stage. However, the reproducibility of the studies is limited due to the lack of a general consensus in the use of some terms. This problem also makes difficult the communication and understanding. For instance, for some important dermatopathologists Bowen disease (BD) is considered a synonym of in situ squamous cell carcinoma. (8) As a consequence, the term BD is used indistinctively for the real BD, bowenoid papulosis, high grade HPV-related squamous intraepidermal lesion (HSIL) and bowenoid actinic keratosis. Nevertheless, these entities not only present clear-cut pathogenic, epidemiologic, clinical and dermatoscopic differences, but also strikingly different prognosis. The risk of metastases after invasive transformation is much higher (30%) for the real Bowen disease than for the other bowenoid lesions. At the microscope, preservation of the basal layer at the earlier stages, the presence of glomeruloid vessels in the upper dermis and, sometimes, the existence of amyloid deposition are the hallmarks of the authentic BD. Another intraepidermal squamous neoplasia, actinic keratosis is attracting the attention of dermatologists, dermatopathologists and researchers due to its high prevalence and the risk of becoming an epidemic health problem, as the patient's life expectancy increases. The concept of actinic cancer field also is now accepted by all medical and scientific community, but it is almost never mentioned specifically in the pathology reports and there is no agreement in the way of managing it.

Finally, genetic aberrations characteristic of malignant neoplasia have been demonstrated in completely benign processes, such as seborrheic keratosis. (9) This shocking findings seem to bring into question the current the understanding of mutational landscapes in human neoplasia. Nonetheless they can play a key role to solve their up to date mysterious behavior. Additionally, it cast doubts on the specificity of mutated DNA detection in serum when using liquid biopsy techniques for cancer screening.

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L 13

UTILITY OF EX VIVO DERMOSCOPY WITH DERM DOTTING IN ROUTINE DERMATOPATHOLOGY.

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Introduction

Currently, the majority of pathology laboratories process skin biopsies without access to clinical and/or dermoscopic images. Macroscopy of skin tumors is usually limited to a quick visual inspection, measurement of the lesion, standard transverse sectioning and random cutting at 2 to 3 different levels. Since the introduction of dermoscopy in the follow-up of pigmented skin lesions, focal areas that are only visible with dermoscopy, may be the reason for diagnostic excision of lesions. Communication of this information to the pathologist may assist in the correct examination of these lesions[1]. It has been shown that routine diagnostic procedure only submits 2% or less of the excised tissue for examination[2]. Especially for pigment tumors, this is a worrisome fact as one can imagine that areas of concern visible for the clinician can be missed by the pathologist when these areas are not discernable for the naked eye. Blind transverse sectioning can transect these areas and as a consequence they are lost for proper evaluation.

Ex vivo dermoscopy with derm dotting : method

In 2007, Scope et al. described the added value ex vivo dermoscopy (EVD) can give to the pathologist by guided tissue sectioning[3]. Since 2011, we systematically use EVD with derm dotting (DD) in our dermatopathology lab. With this simple and easy method, focal areas or suspected section planes observed through a polarizing dermoscope after excision and fixation of the lesion, are marked with nail varnish. For this "derm dotting", we use standard nail varnish which dries very fast and is applied with a brush for larger marks or with a tooth pick to make smaller dots. The varnish has proven to be resistant to tissue processing and permits the technician to orientate the specimen and cut into the diagnostic zone of interest. Under the microscope, the marked zone is recognized as a thick brown gray granular plaque on the horny layer with a thickness of 2–3 mm. Varnish is applied on black dots, areas of depigmentation, structureless zones, ulcerated areas and narrow margins. Several colors can be applied which can be distinguished under the microscope. The lab technician (trained for EVD) also makes a drawing of the lesion with indication of varnish application which enables the pathologist to easily identify and investigate the areas of interest under the microscope. This new method of derm dotting was described by our lab in 2013[4]. Others have afterwards implemented this method in the examination of pigment lesions[5]. The technique is simpler than the micropunch technique developed by Braun et al.[6] which was recently used in combination with IVD by Merkel et al.[7] The use of a 1-mm micropunch creates an artifact in the biopsy that interrupts the histological picture and that can result in problems in the handling of the tissue section. The technique of EVD with DD as we apply, does not interfere with the quality of the histology of the underlying lesion. Although derm dotting could be done by the referring dermatologist just before the resection of the lesion, we prefer to have the intact dermoscopic picture and by application of ex vivo derm dotting decide ourselves which areas will be marked.

INVITED LECTURES

Ex vivo dermoscopy : comparison with in vivo dermoscopy

We investigated the differences between in vivo and ex vivo dermoscopy by comparing the images of more than 100 IVD and EVD images of skin tumors from a private dermatology practice. All images were scored independently by four observers (3 dermatologists and 1 dermatopathologist) who were blind to the histopathologic diagnosis and analyzed for the presence of colors, structure and vessels[8].

We observed that the ex vivo image gives a darker image compared to the IVD image with the appearance of new blue in 32.2%, white in 24.7% and loss of red in 70.1% of cases. Most structures were well preserved and new structureless and crystalline areas were seen in respectively 19.3% and 16.8% of cases. Squamous structures and crusts were lost in 13.9% and 10.6% of cases. Blood vessels disappeared in 67% of cases.

These findings demonstrate that the EVD image can be considered as helpful tool in dermatopathology to give direction to targeted tissue processing and examination of skin tumors.

Ex vivo dermoscopy with derm dotting : comparison of diagnostic performance with standard method of skin biopsy processing

We recently evaluated the impact of using EVD with DD on the reliability of pathological margin evaluation, accuracy of diagnosis and appropriate staging of skin tumors by comparing 6526 skin biopsies examined with standard method of processing, to 8584 biopsies examined with EVD and DD11. The biopsies that were processed using the standard method were analyzed in a hospital-based general pathology laboratory, the biopsies processed with EVD and DD were done in a private dermatopathology laboratory. Biopsies from both periods were diagnosed by the same dermatopathologist (MH). We observed that the use of EVD with DD significantly increases the detection of positive section margins in non-melanoma skin cancer from 8.4% to 12.8%. The most significant increase was seen in Bowen's disease , invasive squamous cell carcinoma (ISCC) and superficial type of basal cell carcinoma (BCC) . By application of EVD and DD, a specific clinicopathologic diagnosis could be made in 25.8% of nevi compared to only 9.0% using the standard method of processing. The incidence of moderately and severely dysplastic nevi increased from 1% to 7.2% and 0.6% to 1.4% respectively. We also noticed a significant increase in the detection of ulceration in melanomas ≥ 1 mm (24% to 31%), the number of nevi-associated melanomas (15.5% to 33.3%) and the number of collision lesions (0.07% to 1.07%). The median run through time for nevi decreased from 2 days to 1 day, for melanomas from 5 days to 2 days and for BCC from 2 days to 1 day.

Ex vivo dermoscopy with derm dotting : use as a scientific tool

EVD with DD is furthermore an interesting scientific tool and ideal method to further identify the histopathologic correlates of dermoscopic structures and colors. Like IVD, EVD is informing the pathologist in a horizontal way of different tissue structures at different depths of the skin. After transverse sectioning and cutting, the vertical information is revealed to the pathologist. For most structures and colors, this horizontal-vertical comparison can identify the morphologic substrate of the EVD observation. For structures like shiny white streaks, the horizontal information alone is not sufficient. With superficial transverse cuts of the residual tissue however, a mirror image of the dermoscopic picture can be obtained. With this method we were able to identify the histopathologic correlate of rosettes, namely intra osteal keratin and areas of perifollicular fibrosis, confirming that rosettes are a non-lesion specific optical phenomenon.[9]

Ex vivo dermoscopy with derm dotting : application in oral pathology

Derm dotting has also proven to be an inexpensive, simple method that can replace the stitching technique used by surgeons to orient specimens during oral surgery for squamous cell carcinoma[10]. Stitches need to be removed by the pathologist, therefore possibly creating an artifact in the biopsy. The varnish dots or lines can be used to orient the specimen and to easily mark suspect borders or areas of interest to be examined by the pathologist. With derm dotting, the pathologist receives a more

representative slide enabling a more accurate clinicopathological correlation.

Conclusion

Our studies indicate that EVD with DD and thereby adapted cutting of skin tumors, permits a more accurate histologic diagnosis. It allows better margin evaluation, a more profound understanding of lesional architecture and specific evaluation of remarkable/suspicious areas either described by the clinician or observed by the lab technicians. Furthermore, the method is time-saving and easy to implement in a dermatopathology setting. EVD with DD has proven to be a valuable new scientific tool to visualize the clinicopathologic correlation. It enables the dermatopathologist to better understand the lesions and their dermoscopic aspect leading to more confidence in the diagnosis. This individualized skin biopsy approach, demands an intensive communication between the dermatopathologist and the technical staff, thereby improving the education of the technicians who have an important role in the diagnostic process. Therefore, we are encouraged and convinced that it is time for the pathologist involved in tumoral skin evaluation and their technical staff to learn dermoscopy and replace random transverse cutting by lesion-specific and DD guided cutting.

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INVITED LECTURES

L 14

LIQUID BIOPSY IN THORACIC ONCOLOGY BEST PRACTICES: THE TIME IS COMING FOR PATHOLOGISTS.

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Although tumor tissue is still the gold standard source for clinical molecular analyses, cancer-derived material circulating in the bloodstream has become an appealing alternative showing promise to overcome some of the challenges described above. Thus, "liquid biopsy" is the term coined to describe such diagnostic procedures performed on cancer-derived material captured in a blood sample. There are several sources of tumor material that can be assessed by liquid biopsy: cell-free or complexed nucleic acids including circulating cell-free DNA (cfDNA), of which a subset represent circulating tumor DNA (ctDNA), cell-free RNA (cfRNA), and circulating tumor cells (CTCs). cfDNA is composed of small fragments of DNA that are not associated with cells or cell fragments, originating from apoptotic and necrotic tumor cells but also from normal cells that are released into the bloodstream. It is evident that a liquid biopsy holds several advantages over a tissue biopsy in providing minimally invasive personalized treatment and improving the follow-up of NSCLC patients in the clinical setting. However, validation is still needed to enable the enlargement of all the potential applications of liquid biopsies before widespread use in routine clinical practice in lung oncology.

Liquid biopsies open up the perspective of short-term monitoring metastatic cancer patients by way of evaluating the efficacy of treatment and/or early detection of secondary mutations of resistance to treatment. Within this context the pathologist, who has already been required in recent years to take interest in the domain of molecular pathologies of tumors, now faces new crucial challenges. The level of investment and attitude of the pathologists to the practice of liquid biopsies, and thus to the management of techniques of molecular biology from blood samples needs to be urgently considered. No matter what the degree of implication of the pathologist in this new domain, it is mandatory that oncologists, biologists, geneticists and pathologists work together to coordinated the procedures and analyses of tissue or liquid samples from the same cancer patient. Different challenges are now present for the oncopathologists in this new domain of activity. It is pivotal to control the delay in obtaining the results so as to rapidly optimize the appropriate targeted treatment. Finally it is mandatory to setup appropriate procedures for the management of the reception and analysis of the different samples for pathology and/or molecular biology based evaluation.

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L 15

THE 2016 WHO CLASSIFICATION OF TUMORS OF THE CNS: WHAT ARE THE MAJOR CHANGES?

D. Figarella-Branger

The 2016 WHO classification represents a paradigm shift for pathologists who will have to make an "integrated" diagnosis for several tumor types (1, 2). Indeed, the pathologist will have to combine molecular data with classic histological phenotype and grade to make a diagnosis. In this upgraded classification, the term NOS "Not Otherwise Specified" has been introduced; it indicates that the information is insufficient to enable a more accurate diagnosis, or that genetic tests have not been made or have been made incompletely, or that the results do not show the expected genetic alteration.

The 2016 WHO classification also incorporates new entities that are defined by both histology and molecular features such as: diffuse midline glioma, H3 K27M–mutant; RELA fusion–positive ependymoma; embryonal tumour with multilayered rosettes, C19MC-altered and solitary fibrous tumor and hemangiopericytoma (SFT/HPC) which are now considered as a single entity.

It proposes the introduction of new variants such as epithelioid glioblastoma and it recognizes a grade III anaplastic xanthoastrocytoma. It also proposes to remove entities such as cerebral gliomatosis or PNET (Primitive NeuroEpithelial Tumor).

The major changes in this upgraded 2016 WHO classification are observed for glial tumors and embryonal tumors.

Regarding the first one, we can point out the following changes:

1. Unlike the 2007 WHO classification that classified gliomas according to the major cell type (astrocytic, oligodendroglial and mixed), the 2016 WHO classification distinguishes diffuse gliomas from other gliomas. Thus, three categories are described: "Diffuse astrocytic and oligodendroglial tumors", "Other astrocytic tumors" (pilocytic astrocytomas, subependymal giant cell astrocytoma and pleomorphic xanthoastrocytoma) and "Other gliomas" (chordoïd glioma of the third ventricle, angiocentric glioma and astroblastoma).

2. Infiltrating adult gliomas are classified according to two molecular alterations: IDH1/2 mutations and 1p/19q codeletion.

3. The mixed gliomas group almost disappears and it is strongly recommended not to make this diagnosis. If this terminology is used, it will only be with NOS. Indeed, recent studies (3) showed that mixed gliomas had either an astrocyte-like profile (IDH-mutated and 1p/19q-intact) or an oligodendroglial-like profile (IDH-mutated and 1p/19q-codeleted). If the molecular profile is discordant or if these alterations have not been analyzed, these tumors will be diagnosed as NOS mixed gliomas.
Regarding embryonal tumors the most important change is that this upgraded classification lists "genetically defined" and "histologically defined" variants of medulloblastomas, allowing to make diagnosis by a double entrance, genetic or histologic.

The 2016 WHO classification shows some limitations regarding the grading of diffuse gliomas, but it represents a substantial step towards the 5th edition of the WHO classification.

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INVITED LECTURES

L 16

MOLECULAR PROFILE OF GLIOBLASTOMA: CLINICAL APPLICATIONS.

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Glioblastomas (GBM) are the most common malignant primary brain tumor in adults. Due to their invasive nature and to their poor response to standard treatment, they are associated with short survival and uniformly fatal outcome. The genetic heterogeneity of GBM has made the molecular characterization of these tumors an area of great interest and different studies using the most recent advances in genomic technology have revealed the existence of several subtypes within the GBM entity. While consensus has not been reached on the precise nature and means to sub-classify GBM, two to four major subtypes seem to emerge from these different studies. Interestingly, these different subtypes are associated with different prognoses and present different response to therapy. However, due to the fact that such high-throughput analyses are not easily applicable in clinical daily practice, this subclassification is not use in clinics today and is not yet incorporated in the latest WHO brain tumor classification of 2016.

We are thus investigating alternative approaches based on techniques such as immunohistochemistry and targeted Next Generation Sequencing (NGS) to approach this molecular classification. For this purpose, several markers, selected thanks to the hightroughput genomic studies, such as EGFR, PDGFRA, YKL-40, Vimentine and p53 were evaluated by immunohistochemistry on retrospective cohorts of formalin-fixed paraffin-embedded (FFPE) GBM. Moreover, DNA from these GBM was subjected to targeted NGS with the AmpliSeq Cancer Hotspot Panel, using the Ion Torrent platform, which allowed us to analyze known cancer-related mutations in 50 genes and copy number variation for 22 genes.

This approach has the advantages to be easily applicable on daily practice using FFPE samples and to allow large scale-clinical application. Using these techniques we were able to isolate different clinically relevant molecular subtypes of GBM associated with prognosis or treatment response. Moreover, the different genetic alterations identified can serve as diagnostic, prognostic, and predictive biomarkers for tumor classification, patient risk stratification, and targeted therapies.

L 17

UPDATE ON PITUITARY TUMORS.

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I. Introduction

Tumors of the pituitary gland and sellar region represent approximately 15% of all brain tumors [1]. Several categories of tumors may involve the sellar region, reflecting its complex anatomy. Table I lists the most frequent tumors arising in this region. The most common tumors are, by far, the pituitary adenomas representing the third most common primary intracranial tumor in neurosurgical practice, outnumbered only by gliomas and meningiomas [1].

In this lecture, we will review the main changes on the WHO classification of tumors of the pituitary region emphasizing histopathological and molecular genetics aspects of pituitary neuroendocrine (i.e. adenomas) and non-neuroendocrine tumors involving the pituitary gland.

II. Pituitary Neuroendocrine Tumors

1. Introduction

The majority of pituitary neuroendocrine tumors are pituitary adenomas; pituitary carcinomas are a rare condition, accounting for only 0.1 to 0.2% of all pituitary neuroendocrine tumors [2]. Adenomas are benign neoplasms confined to the sella turcica; however, invasive adenomas are frequent [3]. Adenomas may sometimes occur in the sphenoid sinus when they are designated as "ectopic" pituitary adenomas [4].

Pituitary adenomas predominantly affect females between the third and sixth decades; however, no age group is spared [1]. Adenomas are uncommon in the pediatric population and most tumors of childhood are clinically functioning adenomas [5]. Incidental adenomas can be found in nearly 14% of autopsied patients [6].

2. Classification of Pituitary Adenomas

Pituitary adenomas are clinically classified in clinically functioning adenomas and the clinically non-functioning adenomas, according to whether or not an endocrine syndrome is present. The majority of adenomas are functioning tumors and include Prolactin (PRL)-secreting, Growth Hormone (GH)-secreting, Adrenocorticotropic Hormone (ACTH)-secreting, and Thyrotropin-Stimulating Hormone (TSH)-secreting adenomas [6]. About a third of pituitary adenomas are unassociated with either clinical or biochemical evidence of hormone excess [7]. This group includes the gonadotroph adenomas that produce Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH), and the null-cell adenomas. These clinically non-functioning adenomas commonly present with symptoms related to local mass effect such as headaches, neurologic deficits in the cranial nerves including visual field disturbances, and mild hyperprolactinemia due to pituitary stalk compression (the so-called stalk effect). In addition, adenomas may be designated as "silent adenomas" when patients do not show any clinical signs or hormonal elevation in the serum but the tumor demonstrates hormone production by immunohistochemistry.

Pituitary adenomas are histopathologically classified by the World Health Organization (WHO) classification according to the hormone content of the tumor cells as assessed by immunohistochemical stains [8] (Table II). This immunohistochemical classification provides significant information for clinical practice. Moreover, the WHO classification recognizes the role of transcription factors (Pit-1, SF-1, Tpit, etc.) in tumor differentiation according to cellular lineage(s), in regulation of specific pituitary hormones, and in possible tumorigenesis [9]. Recognizing this new data, the current WHO classification has adopted the pituitary-cell lineage for designation of the adenomas [8].

3. Tumor Grading

In the previous edition of the WHO Tumor Classification of Endocrine Organs (2004) [10], the pituitary neuroendocrine tumors were divided into typical adenoma, atypical adenoma, and carcinoma. The majority of pituitary neuroendocrine tumors are typical adenomas with bland histological features, infrequent mitotic figures, and a low Ki-67 proliferative index (less than 3%). Atypical adenomas were defined as adenomas with histological features suggestive of an aggressive clinical behavior including elevated mitotic index and a Ki-67 labeling index greater than 3%, and overexpression of the p53 protein by immunohistochemistry [10]. Using these criteria, the incidence of atypical adenoma is relatively variable (2-15%) [2,11,12], and its prognostic value not yet established despite more than 10 years of classification. In the upcoming WHO classification [8], the term of atypical adenoma is no longer recommended. However, assessment of the tumor proliferative potential by mitotic count and Ki-67 index, in addition to other clinical parameters such as tumor invasion (by MRI studies and/or intra-operative impression), is strongly recommended in individual cases for consideration of clinically aggressive adenomas [13-15].

The classification also recognized some subtypes of adenomas that show an aggressive behavior and patients with such tumors should be carefully followed clinically. These include the sparsely-granulated somatotroph adenoma, the Crooke's cell adenoma, the silent corticotroph adenoma, and the plurihormonal Pit-1 positive adenoma (previously known as silent subtype III).

The WHO classification reiterates the definition of pituitary carcinomas as tumors demonstrating metastatic spread by either craniospinal dissemination or systemic metastases [16]. Pituitary carcinomas are very rare, comprising less than 1% of all pituitary neoplasms [2]. The majority of carcinomas are hormonally active tumors; the most common are PRL-secreting tumors, followed by ACTH-secreting [17,18]. Clinically non-functioning carcinomas constitute about 15-20% of the cases including gonadotroph, silent corticotroph, and rarely null cell carcinomas [17,18]. There are no morphologic criteria to distinguish locally aggressive or even markedly atypical adenomas from pituitary carcinomas when the tumor is still confined to the sella turcica. Morphologic features associated with malignancy including hypercellularity, nuclear and cellular pleomorphism, increased mitotic activity, necrosis, and dural/bony invasion are commonly present but are not necessarily diagnostic of carcinoma.

The mechanisms of progression of pituitary adenoma to carcinoma are not yet totally understood. Although most of the pituitary adenomas are benign tumors, they are frequently invasive into the adjacent structures leading to high level of recurrence. A proliferative continuum from benign adenoma to invasive adenoma to carcinoma has not been demonstrated in the great majority of tumors. The propensity of pituitary adenomas to infiltrate locally and invade adjacent structures appears to be independent of histologic features of the tumors, in that the great majority of invasive adenomas do not progress to carcinomas. Although both clinically functioning and non-functioning adenomas may present as invasive tumors, gross invasion appears to be more frequent in clinically functioning tumors [3], a trend also seeing in pituitary carcinomas [17,18,19].

II. Pituitary Non-Neuroendocrine Tumors

1. Introduction

Although much less frequent than pituitary adenomas, non-neuroendocrine tumors arising in the pituitary are intrinsic tumors of the gland that are important in the differentiation diagnosis of sellar masses (Table I). The main entities considered in this group of tumors and discussed in this lecture are the pituicytomas, the granular cell tumors and the spindle cell oncocytomas.

All three tumors are low-grade, non-neuroendocrine neoplasms of the sella that can clinically and radiologically mimic non-functioning pituitary adenomas. Patient's clinical presentation is mostly related to tumor size, with signs and symptoms of compression of adjacent structures and the pituitary stalk including visual disturbances, headaches, hyperprolactinemia and amenorrhea, and fatigue [20]. Only a minority of the tumors may present with diabetes insipidus [20].

Pituicytoma has been used to describe a number of tumors in the region of the sella including pilocytic astrocytomas and granular cell tumors. In 2000, Brat and colleagues formally defined pituicytoma as a

distinctive low-grade glial neoplasm that arises from pituicytes [21]. Pituicytes are the specialized glia of the neurohypophysis and pituitary stalk that has a sustentacular function in the posterior pituitary.

Granular cell tumors of the pituitary - unlike those tumors located in gastrointestinal tract and skin that are believed to be of Schwann cell origin - are considered to be unique neoplasms of the neurohypophysis and pituitary stalk, and have also been suggested to derive from pituicytes [22].

Spindle cell oncocytomas of the adenohypophysis were first described in 2002 by Roncaroli and colleagues as non-endocrine neoplasms characterized by mitochondria-rich, spindle to epithelioid cells [23]. Although these authors have proposed a folliculostellate cell origin based on similarities of their ultrastructural and immunohistochemical features [23], recent data on the shared TTF-1 immunoreactivity of pituicytomas, granular cell tumors and spindle cell oncocytomas [24,25] may suggest more similarity in origin than was originally suspected.

The specialized glia of the neurohypophysis and pituitary stalk, the pituicyte, is embryologically derived from the floor of the diencephalon. Recent data have shown that the floor of the diencephalon is an area under the influence of the thyroid transcription factor-1 (TTF-1), a transcription factor known to be critical for the development of lungs and thyroid, and now revealed to play a role in the induction of the infundibular anlage during development of the posterior pituitary and infundibulum [24]. Similar to the developing and normal pituicytes, these three tumors - pituicytomas, granular cell tumors and spindle cell oncocytomas - show diffuse nuclear expression for TTF-1 [24,25]. On the other hand, folliculostellate cells, the sustentacular cells of the adenophypophysis, do not express TTF-1 [24].

Furthermore, the ultrastructural morphology of these tumors shows similarities with the described variants of normal pituicytes, another indication of a possible common pituicyte-lineage [25]. Pituicytes have five distinct ultrastructural variants: light (or major), dark, granular, ependymal and oncocytic [26]. The current hypothesis is that these non-endocrine pituitary tumors would originate from specific variants of pituicytes, i.e. pituicytomas from the light/major variant, granular cell tumors from the granular variant, and the spindle cell oncocytomas from the oncocytic variant [22,25,27,28].

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L 19

MOLECULAR DIAGNOSTICS IN KIDNEY CANCER.

Antonio Lopez-Beltran

Renal cell carcinoma (RCC) is the most common renal malignancy, with three common subtypes representing about 95% of all renal tumors: clear cell (ccRCC, 75% of RCC), papillary (pRCC, 10%), and chromophobe (chRCC, 5%). The fourth most common renal epithelial tumor is oncocytoma (OC, 5%), a benign neoplasm whose differential diagnosis from subtypes of RCC with cytoplasmic eosinophilia is challenging in daily practice.

Primary renal tumors comprise an interest¬ing and heterogeneous group of entities with diverse characteristics and variable clinical out¬comes. Understanding their molecular and genetic features is important, as prognosis can vary greatly between neoplasms that appear sim¬ilar with other methodologies. Light microscopy has long been utilized as the main classification tool, and it remains highly useful and specific today. However, modern techniques have begun to demonstrate utility in resolving the differ¬ential diagnosis of histopathologically similar lesions as well as in defining altogether new ones. Utilization of molecular and cytogenetic tech¬niques has improved our understanding of the mechanism of tumor initiation and progression, creating new possibilities for both diagnosis and prediction of outcome/therapy response. Nonetheless, some authors have had success in correctly clas¬sifying renal tumors based solely on molecular methods, such as loss of heterozygosity (LOH) analysis or array-based comparative genomic hybridization (CGH). Likewise, FISH, classical cytogenetics and karyotyping are methods that have been studied extensively in kidney neoplasms.

Each of the most common histologic subtypes harbors specific recurrent genetic abnormalities, such as deletion of 3p in ccRCC, trisomy 7 and 17 in pRCC, multiple monosomies in chRCC, and a nearly diploid genome in benign oncocytomas.

Most renal cell tumors are sporadic, however, there are four main hereditary cancer syndromes involving the renal epithelium: von Hippel Lindau (VHL) syndrome, which predisposes to development of ccRCC; hereditary papillary renal cell carcinoma (HPRCC); Birt-Hogg-Dube syndrome (BHDS), which predisposes to development of chRCC and OC; and hereditary leiomyomatosis and renal cell carcinoma (HLRCC), which predispose to pRCC and collecting duct carcinomas.

Investigation of the Mendelian single-gene syndromes, such as von Hippel

Lindau syndrome (VHL: VHL gene), hereditary papillary renal cell carcinoma (HPRCC: MET gene), Birt-Hogg-Dube (BHD: BHD gene), and hereditary leiomyomatosis renal cell carcinoma (HLRCC: FH gene), however, have provided an

opportunity to develop pathway-specific therapies

Proper diagnosis of RCC is critical to ensure appropriate patient management because each subtype of renal epithelial tumor has a specific recurrence risk and treatment regimen. The 5-year survival and disease-free progression of renal cell carcinomas varies by subtype: chRCC (100 and 94%), pRCC (86 and 88%), ccRCC (76 and 70%), RCC unclassified (24 and 18%) (5). Oncocytomas are benign neoplasms with no risk of metastasis, but they can morphologically mimic some renal cell carcinomas and are often part of the differential diagnosis. Cytogenomic profiles obtained from

array-based studies can be useful in the evaluation of diagnostically challenging renal epithelial tumors, even in cases where FISH and IHC yield ambiguous results. SNP arrays could resolve 94% of morphologically challenging renal tumors. In addition, the genetic diagnosis of renal tumors by comparative genomic hybridization on FNA biopsies can improve differential diagnosis in particular with limited tissue samples.

Surgical resection is generally indicated in patients with a solid renal mass to determine if the mass is malignant. Surgery, if performed, provides not only diagnostic tissue, but in most cases also definitive therapy.

In addition to assisting in correct classification, the ability to identify tumors within each subtype that are most likely to recur would allow management strategies to be further tailored to each patient's risk. For ccRCC and pRCC, the strongest predictors of survival are tumor stage, nuclear grade, and necrosis. However, there is currently no reliable biomarker that can predict metastatic or local recurrence in patients with organ confined tumors (stages I and II), and the significance of histologic subtype for prognosis is not independent of stage. Cytogenomic profiles can assess the diagnostic genomic changes as well as genomic changes associated with prognosis. For example, detection of a 3p deletion in a morphologically challenging renal tumor would support the diagnosis of ccRCC because nearly 100% of ccRCC demonstrate this feature. In

addition, a cytogenomic profile could detect the deletion of 9p or 14q, both associated with poor prognosis (Table 2). This information could allow surgeons and oncologists to design appropriate monitoring and interventions for those tumors that are more likely to recur, such as changing monitoring practices or the use of adjuvant treatments.

The emergence of targeted therapies, such as antiangiogenic drugs and mTOR inhibitors, has dramatically improved the progression-free survival (PFS) of patients

affected by ccRCC, and is gradually improving OS. Understanding of the VHL-HIF-VEGF cellular hypoxia pathway has provided the foundation for the development of treatment strategies based on tyrosine kinase inhibitors (TKIs) with activity against VEGF receptors, such as sunitinib and sorafenib. Likewise, MET mutations are seen in hereditary and sporadic pRCC, and clinical trials targeting the MET pathway are ongoing. Preclinical studies are underway targeting the BHD gene pathway for BHDS and sporadic chRCC. At this moment, there is no evidence that

mutational status on VHL or MET genes modifies a tumor's response to targeted agents. However, it is possible that as more specific targeted drugs are developed, the evaluation of mutational status might be necessary to qualify for therapy.

Assessing the cytogenomic profiles of renal epithelial tumors allows for diagnostic support, refined prognostic information, and can guide appropriate choice of therapy.

There are several techniques in use in clinical and research laboratories to assess chromosome copy number and/or LOH status, including conventional cytogenetics, FISH analysis, microsatellite LOH analysis, and array-based genomic analysis (aCGH or SNP arrays). The great majority of diagnostic and prognostic alterations can be detected with any of these approaches. Each technique has strengths and limitations,

however, and these differences may explain some of the discrepancies reported in the literature regarding frequency of chromosomal imbalances, and should be kept in mind when guiding clinical testing strategies and evaluating diagnostic results. Conventional cytogenetics is the method of choice when assessing for non-recurrent balanced translocations, such as those present in familial non-VHL ccRCC. FISH can

be used to detect the presence of recurrent chromosomal translocations, such as those present in pediatric tumors involving the Xp11.2 locus. Array-based genomic analysis is

quickly becoming the method of choice for comprehensive evaluation of chromosomal imbalances in kidney and other solid tumors.

Variability in the frequency of chromosome copy number and LOH status in renal tumors reported in the literature may be due to limitations in different assays. In addition, variability may also arise from improper data normalization in tumors with hyperdiploid or hypodiploid genomes when using array-based techniques.

The rapid development of NGS technologies may allow the simultaneous analysis of mutational status, translocation events, and chromosome copy number changes with a single technology in the near future. Although not specifically applied to renal tumors, promising advances in copy number analysis with NGS have been shown to be useful in paraffin-embedded tissues and, thus, could enable the routine clinical utilization of this approach.

L 20

NEW AND EMERGING ENTITIES IN KIDNEY CANCER.

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The Vancouver consensus conference of the International Society of Urological Pathology (ISUP) provided the foundation for much of the 2016 World Health Organization (WHO) renal tumour classification.

Within the clear cell renal cell carcinoma (RCC) group, multi-cystic clear cell renal cell carcinoma is considered as a neoplasm of low malignant potential. Subtyping of papillary renal cell carcinoma is of value and the oncocytic variant of papillary renal cell carcinoma should not be considered as a distinct entity. The hybrid oncocytic chromophobe tumor which is an indolent tumor that occurs in 3 settings, namely Birt-Hogg-Dubé Syndrome, renal oncocytosis and as a sporadic neoplasm, is placed within the chromophobe renal cell carcinoma group.

Five entities are recognized as new distinct epithelial tumors: tubulocystic renal cell carcinoma, acquired cystic disease associated renal cell carcinoma, clear cell papillary (tubulopapillary) renal cell carcinoma, the MiT family translocation renal cell carcinomas (in particular t(6;11) renal cell carcinoma) and hereditary leiomyomatosis renal cell carcinoma syndrome-associated renal cell carcinoma.

There are three rare epithelial carcinomas that are considered as emerging or provisional new entities: thyroid-like follicular renal cell carcinoma; succinic dehydrogenase B deficiency associated renal cell carcinoma; and ALK-translocation renal cell carcinoma. Further reports of these rare entities are required to better understand the nature and behavior of these highly unusual tumors.

While the morphotype of RCCs is usually discernible on examination of the H&E stained tissue section, markers are often useful for confirming diagnosis in difficult cases. The main subtypes of RCC (clear cell, papillary, chromophobe and collecting duct RCC) each have typical immunostaining profiles and as such immunohistochemistry is of diagnostic utility for these tumors. Immunohistochemistry stains can also be used to distinguish malignant tumors from benign tumors and tumor-like processes, and can also be used to determine if renal tumors are of metastatic origin. In routine clinical practice of genetic studies this is still of limited value. Fluorescence in situ hybridization should be undertaken to confirm a diagnosis of translocation carcinoma, if the diagnosis is suspected morphologically or if the patient is under 30 years of age. Other differential diagnoses where genetic studies were considered valuable were; mucinous tubular spindle carcinoma and papillary RCC with sarcomatoid areas; and clear cell (tubulo) papillary RCC and either clear cell or papillary RCC.

The RCC classification has been expanded in recent years and a modified WHO classification is now in use. In the era of targeted therapy, the fact that different histotypes have different outcome and different response to therapy represents a major advance. Introduction of modern percutaneous biopsy protocols in clinical practice allows histologic diagnosis in the majority of cases. This methodology is also suitable for molecular diagnostic purpose and represents a changing paradigm in RCC.

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INVITED LECTURES

L 22

SEROSAL CYTOPATHOLOGY: MORPHOLOGICAL ASPECTS. GAND 2016.

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Cytopathological diagnosis of serous effusions can provide major information for the management of patients presenting with effusions. A reliable differentiation between malignant and reactive effusions, leading to a reliable diagnosis of carcinomatous effusions is of high clinical relevance. The finding of tumour cells in such a specimen signifies that the patient has an often incurable cancer by surgery.

Cytology plays also a central role in the aetiological diagnosis of serous effusions. The identification of the primary site of metastatic tumours is of paramount importance for adequate treatment. Clinical features such as age, sex and often dissemination pattern of the tumour, give a first indication.

Cytological features of malignant tumours in pleural, pericardial and peritoneal effusions are very similar. Cytologically, it is possible to define four principal types of tumours in effusions: adenocarcinomas in about 60%, squamous cell carcinomas in about 5%, non small cell carcinomas in about 30%, small cell carcinomas in about 1%, and other tumours in about 4%. The vast majority of metastatic malignancy in effusions provides from a restricted number of primary tumours.

Many tumour markers have been developed in the past two decades as immunohistochemical aids in the differential diagnosis of malignant tumours. Some antibodies specifically help to identify the origin of the tumours. Differents panels of antibodies have been tested to determine whether expression of specific tumour-related antigens could predict the origin of the tumours. The choice of antibodies is still first determined by the inital cytological diagnosis.

The aim of this presentation is to present the major cytomorphological criteria for the diagnosis of metastases and mesotheliomas in effusions.

Ancillary techniques

1 Sampling techniques

- Serous effusion is usually sampled by the simple procedure of inserting a needle into the fluid-containing body cavity ; peritoneal fluid is removed by abdominal paracentesis, pleural fluid by thoracocentesis and pericardial fluid by pericardiocentesis.
- Serous effusion may be sampled at the time of surgical exploration.
- Peritonel washings are obtained by instilling physiologic saline solution into the various zones of the peritoneal cavity in patients undergoing abdominal exploration for gynecologic neoplasms.

However, the last two methods of sampling may induce morphological distorsion of the mesothelials cells.

2 Preparation of the specimens

Several preparatory methods may be are used in processing effusions. Regardless of the preparation method used, slides are most often stained using both the standard Papanicolaou and the MGG or Diff Quick stains.

- Smears: centrifugation of 10 ml at 1500 rpm for 5 minutes. The supernatant is resuspended in 0,5 ml of the supernatant and smeared. Air-dried and MGG staining and/or after ethanol fixation, Papanicolaou staining
- Cytospin preparations are stained by MGG or Diff Quick/toluidine blue stains.
- Liquid-based cytology such as Thinprep-processed slides is stained by Papanicolaou
- Cell block technique on the residual sediment requires HE staining.

3 Immunocytochemistry (ICC) is a useful method complementary to cytopathologic diagnosis.
ICC is being used increasingly as an adjunct to conventional cytomorphology.

- ICC can be performed on smears prepared from effusions

Papanicolaou-stained smears may be destained with 0,5 hydrochloric acid in 95% ethanol and rinsed in tap water for 5 min

Air-dried smears, 2 to 4-days old smears stored at the room temperature. The smears may be fixed for 10 minutes in 4° acetone, dried and then rehydrated in phosphate -buffered saline before incubation.

- ICC can be performed on paraffin-embedded samples from effusions

Cell blocks preparation: cell block preparation methods differ from institution to institution

For paraffin embedding by centrifugation or filtration

Thromboplastin plasma cell block technique

Which method must we choose ?

Application of ICC to conventional smears has the following limitations :

- Limited cellularity for testing
- Specific staining may not be obtained because of disrupted cells and membranes fragments sticking to slides
- Lack of parallel samples of the same cells for additional tests or to run the control
- Large area of the conventional smears need to be covered leading to wastage of antibodies

For some authors cell blocks provide the best morphologic interpretation. For others cell blocks contain small numbers of isolated malignant cells, and artifactual cell distorsion may cause pitfalls. (7) They assess superior results with smear preparations.

4 Antibodies panel. A number of antibodies have been applied to serous effusion samples with varying degrees of efficacy.

4.1. Mesothelial markers

Mesothelial markers are commonly expressed in mesotheliomas but not in carcinomas and in sarcomas.

Calretinin is a calcium-binding protein that is expressed in a variety of normal tissues such as mesothelial cells, adipocytes, neural tissue and sex cord tumours.

Only antibodies against human recombinant calretin have proved to be useful in the diagnosis of mesothelioma. The staining pattern is cytoplasmic with peripheral condensation. The combination of both nuclear and cytoplasmic staining is the more specific for mesothelial differentiation.

Calretin is regarded as being **the most sensitive and one of the most specific of the positive mesothelial markers**. A negative staining for calretinin should be regarded as a strong indication against the diagnosis of mesotheliomas.

Cytokeratin 5/6

This antigen is the first mesothelial marker described by Blobel in 1995. The antibody is the D5/16B4 and the XM26 directed against keratin 5. The staining pattern is cytoplasmic.

Focal staining can explain negative result on biopsy. Positive reaction is found in all mesotheliomas, in all squamous carcinomas, and in one third of serous carcinomas.

WT1 protein

It is one of the most recently recognized positive mesothelioma markers. Nuclear positivity is demonstrated in 45% to 93% of mesotheliomas. WT1 is not expressed in adenocarcinomas and squamous

cell carcinomas of the lung but is expressed in 83% to 100% of the serous carcinomas. It has no utility in distinguishing serous carcinomas from peritoneal mesotheliomas.

All the antibodies (HBME-1, trombomodulin, mesothelin, D2-40; Podoplanin) with different types of staining pattern such as membranous staining in mesotheliomas and cytoplasmic staining in carcinomas are not reliable for immunocytochemistry : the risk of overlapping staining pattern between metastatic carcinomas and mesotheliomas is higher on effusions.

4.2. Positive epithelial markers

Epithelial markers are commonly expressed in carcinomas but not in mesotheliomas.

A large number of positive carcinoma markers have been investigated: P/m CEA ;MOC-31 ; Ber-Ep4 ; B72.3 ; CD15 ….

MOC-31 is an anti-Ep-CAM antibody that has been extensively investigated as an immunohistochemical marker in the diagnosis of mesotheliomas.

Because of its high sensitivity and specificity, MOC-31 is one **the best currently available markers** available for distinguishing between mesothelioma (2% to 10% positivity in scattered tumoural cells) and lung adenocarcinoma (90% to 100% positivity), squamous carcinomas of the lung and serous carcinomas.

Ber-EP4 is the anti-Ep-CAM antibody that has been the most investigated for the diagnosis of mesothelioma. It is useful for distinguishing mesothelioma from lung adenocarcinoma, serous carcinomas and squamous carcinomas of the lung. The specificity and the sensibility of Ber-EP4 is somewhat lower as the specificity and the sensibility of MOC-31.

CEA is the first marker accepted as being useful for the distinction between metastatic adenocarcinoma and epithelioid mesothelioma. pCEA has a high sensitivity and a low specificity. Because of his good sensitivity and high specificity mCEA is still regarded as one of the best immunohistochemical markers betwwen these two malignancies.

CA 19-9 has limited utility. It is highly specific for distinguishing serous carcinomas from mesotheliomas but its utility is limited by its low sensitivity. It has no utility for distinguishing mesotheliomas and lung adenocarcinomas.

EMA is expressed in large variety of epithelial cells. The monoclonal antibody used is the E29. EMA do not stain normal and reactive mesothelial cells on biopsy and surgical specimens.

4.3. Tissue-associated markers have restricted expression in carcinomas and are not expressed in mesotheliomas

Thyroid Transcription factor 1. TTF-1 is a tissue-specific nuclear transcriptor factor expressed in the thyroid epithelial cells and in type 2 pneumocytes of the lung.

The immunostainig of the 8G7G3/1 monoclonal antibody is nuclear.

TTF-1 is expressed in approximately 80% of lung adenocarcinoma. The expression is higher in well differentiated tumour and lower in poorly differentiated and mucinous adenocarcinomas. The main application of TTF-1 immunostaining is to distinguish mesotheliomas which are invariably negative, from lung adenocarcinomas. This antibody allows clear-cut distinction between pleural metastases from a lung adenocarcinoma and pleural metastases from extra-lung adenocarcinoma.

Estrogen and progesterone receptors

Estrogen and progesterone receptors are frequently expressed in serous carcinomas of the ovary and peritoneum. Estrogen receptors are very rarely present in mesotheliomas. Immunostaining for estrogen receptors is useful for discriminating between breast adenocarcinoma and epithelioid mesotheliomas.

Immunostaining for estrogen receptors is useful for discriminating betwween papillary serous carcinoma (positivity for ER in 93% of cases) and peritoneal epihelioid mesotheliomas (always negative).

P63/P40

It is a recently characterized nuclear transcription factor. The latter group of p63 molecules is found

in basal cells of the stratified epithelia, prostate, salivary glands and breast, and in myoepithelial cells. The protein is expressed in squamous carcinoma of various primary sites including the lung (80%-100%). In contrast mesotheliomas rarely express p63.

Renal cell carcinoma (RCC Ma)

The antibody recognizes a 200-kd glycoprotein present in the normal proximal tubule of the kidney. Positivity of RCC Ma for conventional renal cell carcinoma is ranged from 75% to 85%, for papillary renal cell carcinoma from 75% to 95% and for chromophobe carcinoma from 0% to 45%. In contrast mesotheliomas usely do not express RCC Ma. Mestastasis in peritoneal effusion of renal carcinoma are very rare, and RCC Ma has low utility in effusions.

4.4. Cytokeratin (CK) subsets

Cytopankeratins like AE1/AE3 and KL1 are widely express by all types of carcinomas and mesotheliomas and by normal and hyperplastic mesothelial cells.

They have limited utility in effusions.

Immunoreactivity to cytokeratin 7 (CK7) and 20 (CK20) has proven its diagnostic utility due to differential expression of CKs in various epithelia and malignant cells.

CK7+ CK20 - : lung, breast and ovaries
CK7+ CK20 + : urothelial, pancreas, stomach
CK7- CK20 + : colon, rectum
CK7-CK20 - : liver, kidney, prostate

4.5. Mesenchymal markers such as actin, desmin, CD 34, CD 31, CD 10 BcL2 are commonly expressed in sarcomas but not in mesotheliomas. They have no utility for the diagnosis in effusions.

Recommendations. The selection of the markers to be used depends of the type of effusion, the cytological pattern of the tumour cells and the sex of the patient. Markers selection will vary according to the differential diagnosis. In a man presenting with a pleural effusion and with three dimensional clusters of tumoural cells, the most likely differential diagnosis is between metastatic peripheral lung adenocarcinoma and epithelioid mesothelioma. Pleural effusion with tumoural cells occurring in a woman suggests differential diagnosis between metastatic brest carcinoma and epithelioid mesothelioma.

The markers recommended in the common differential diagnoses are detailed below case by case.

Case 1: Malignant mesothelioma (MM)

Clinical data: 64 year-old man. Worker. Chest pain. Baisse de l'état général. Right pleural effusion.

Cytology:

At low magnification highly cellular specimen. Numerous large to giant isolated round tumoural cells ; single cells easily observed at low magnification.

At high magnification smears are characterized by a single-cell population with variation from benign mesothelial cells to malignant higly atypical cells.

The tumoural cells are all of mesothelial type: relatively low N :C ratio -cells with abundant cytoplasm such that the N :C ratio is relatively low- ; cytoplasmic skirts -presence of a cytoplasmic halo around each cell caused by microvilli- ; bubbly cytoplasm -presence of small blebs in the cytoplasm- ; vacuolated cytoplasm; cyanophilic cytoplasm -metachromatic color to the cytoplasm- ; multinucleation; rounded nuclei ; presence of prominent nucleoli ; presence of cellular windows – presence of distinct clearing between two mesothelial cells because of microvilli;

Diagnostic: malignant epithelioid mesothelioma of non cohesive cell type

In cytology 2 subtypes of epithelioid mesothelioma are recognized : the malignant epithelioid mesothelioma of non cohesive cell type and the malignant epithelioid mesothelioma of cohesive cell type. The last cytological type is characterized by highly cellular preparation with abundant 3D clusters, scalloped border around clusters, cellular windows within the clusters and presence of identifiable nucleoli on the cells in a cluster.

Immunocytochemistry is very useful

- Two positive mesothelial markers: calretinin, CK 5/6
- Two negative epithelial markers: CEA, MOC-31

Effusion in malignant mesothelioma :

- 40 % of MM do not present with effusions
- 40% of those with effusions have no cytologic evidence of MM
- Sarcomatoid variant of MM are not prone to develop pleural effusions
- Appropriate immunocytochemistry with a precise description of the cytological features enhances the averal sensitivity of the diagnosis .

Case 2: Metastatic pleural adenocarcinoma of the lung

Clinical data: 72 year-old man ; heavy smoker ; dyspnae ; left pleural effusion

Cytology:

At low magnification: highly cellular specimen

At high magnification: presence of a two-cell population ; large amount of malignant cells occuring isolated or in clusters

Tumoural cells features: relatively high N :C ratio ; smooths cell borders ; single cells with high N :C ratio ; multinucleation ; nuclear enlargement ; vacuolated cytoplasm ; dictinct prominent nucleoli ; anisonucleosis within the sheets ; presence of 3D clusters ;

Many cells show various degrees of vacuolisation.

Cytological classification of lung adenocarcinoma :

1. adenocarcinoma with mucin production
2. adenocarcinoma without mucin production

Well differentiated 3D clusters can be associated with poorly differentiated isolated large tumoural cells with squamous differentiation

Immunocytochemistry: Positive markers : CK7 and TTF1 in about 80% of cases.

The negativity of TTF-1 may not exclude the bronchopulmonary origin of the adenocarcinoma.

Effusion in lung adenocarcinoma

Frequent : the first most common cause of pleural effusion (36%)

They are 5 subtypes of invasive adenocarcinoma of the lungs: lepidic adenocarcinoma; acinar adenocarcinoma; papillary adenocarcinoma; micropapillary adenocarcinoma; solid type adenocarcinoma , according to the last histological classification WHO 2015

Adenocarcinoma of lepidic type cannot be diagnosed in pleural effusion: the tumoural cells are growing along the alveolar walls and are not invading the visceral pleura and the lymphatic vessels. Otherwhise it will be a mixte type adenocarcinoma with a lepidic component combined with an other subtype.

Case 3: Metastatic pericardial adenocarcinoma of the breast

Clinical data: 58 year-old woman with a past history of breast adenocarcinoma cured by surgery , radio and chemotheray 22 years ago. Pericardial effusion with left pleural effusion.

Cytology :

At the low magnification: highly cellular specimen.
At the high magnification: numerous tumoural cells of small to medium size.

Type 1: compact, dense, round, so called spheres. The high optical density of these spheres may not allow to discern the individual cytologic features characteristics of malignancy. Careful examination will reveal isolated adenocarcinomatous cells in the back ground of the smears. Proliferation of spheres are characteristic of duct cell adenocarcinoma of the breast

Differential diagnosis: metastatic adenocarcinoma from others sites

Type 2: numerous small, isolated, round neoplastic cells characterized by fairly uniform shape and size. Individual cells show irregularity of nuclear shape, heavy nuclear membranes and prominent nucleoli. This cytological presentation is related to lobular carcinoma.

Differential diagnosis: small mesothelial cells

Cytoplasm of cells may display cytoplasmic vacuoles lined by microvilli and containing a mass of mucus

Immunocytochemistry :

- Positive markers : CK7 +; Estrogen and progesterone receptors +

High sensibility (ER : 72% ; PR : 52% ; ER+PR : 84%) and less specificity

- Negative markers : Calretinin - ; TTF1-

Normal, hyperplastic and tumoural mesothelial cells never express estrogen and progesterone receptors. Tumoural cells of breast adenocarcinoma never express calretin.

Effusion in metastatic breast adenocarcinoma :

- High frequency of the disease
- High frequency of the metastatic breast cancer in pleural fluid (the second frequent cause 25%), occasionally in ascitic and in pericardial fluids
- First manifestation of recurrent carcinoma
- Important incidence for the therapy

Case 4: Metastatic adenocarcinoma of the ovary

Cytology:

High number of tumoural cells

Large acinar or papillary clusters with numerous tumoural single cells.

Morphological features of an adenocarcinoma. Many of the cells are hypervacuolated.

Vacuolisation may be due to mucin, glycogen or degenerative change.

Immunocytochemistry: CK7-; CK20+

Effusion in ovarian adenocarcinoma

- frequent cause of peritoneal effusion and to a lesser extent pleural effusion

- often first manifestation of the ovarian carcinoma

Case 5: Metastatic gastric adenocarcinoma

Case 6: Metastatic colonic adenocarcinoma

Cytology: Similar cytological features

Few or large number of single tumoural cells many of signet-ring type

Immunocytochemistry:

Metastatic adenocarcinoma of the stomach: CK7+ ; CK20+

Metastatic adenocarcinoma of the colon: CK7- ; CK20+

Effusions in metastatic gastrointestinal adenocarcinoma :

- uncommon event

- peritoneal effusion

Case 7: Metastatic small cell carcinoma of the lung (SCC)

Clinical data: 52 year-old man ; heavy smoker ; mediastinal mass with large mediastinal lymphnodes ; recurrent left pleural effusions.

Cytology

Amorphous clusters and chain of cells. The nuclei are angulated and show more variation in size and shape than those of lymphocytes. Absence of prominent nucleoli.

Tumoural cells do stand out more in the air dried preparation. On air dried MGG staining preparation the cells are larger and show more frequently than by Papanicolaou staining a small amount of cytoplasm.

In cell block preparation the cells frequently show more cytoplasm and the nuclei may return their elongated form as in biopsy.

Differential diagnosis

Many SCC tumoural cells become necrotic producing pyknotic nuclei that simulate lymphocytes.

Lymphomatous cells do not cluster in either small or larger aggregates.

Immunocytochemistry: CK 7+; neuroendocrin markers such chromogranin A + ; TTF1 +

Case 8: Metastatic squamous cell carcinoma (SQCC)

Clinical data

58 year-old man. Squamous cell carcinoma of the pharynx. Right pleural effusion.

Cytology

- Well differentiated squamous cell carcinoma :

Polygonal anucleated tumoural cells ; necrotic background. Malignant pearls.

- Poorly differentiated squamous cell carcinoma :

Polygonal type squamous carcinomatous cells similar in size and shape to an intermediate squamous cells ; however the nucleus is larger and irregular in shape. The chromatin is coarse.

Immunocytochemistry: usually not useful

Effusions in metastatic squamous cell carcinoma

- Metastasis of squamous cell carcinomas are uncommon.
- Primary neoplasms are SQCC of lung, larynx, oesophagus and female genital tract.

Case 9: Metastatic lymphoma

Clinical data: A 58 year-old woman. History of B cell lymphoma. Chemotherapy.

Cytology : Isolated large lymphoma cells mixed with mature lymphocytes.

Large round to lobulated nuclei and abundant deep blue cytoplasm with cytoplasmic vacuolation.

Extensive degenerative/apoptotic changes.

Diagnosis: large B-cell lymphoma CD 20+

Immunocytochemistry : CD 20; CD3; CD30 and LCA

Differential diagnosis :

Lymphocyte-rich effusions: they are similarities between reactive lymphocytes and small cleaed cell and mixed small and large cleaved cell lymphomas

Effusions in lymphoma

- Frequent : the third most common cause of malignant pleural effusion (more than 10%)
- Peritoneal effusion
- In almost every case the patient is known to have the disease
- Rarely finding of lymphoma cells in an effusion is the first manifestation of the disease
- Cytomorphological features supported by immunocytochemical studies may be useful for the classification of lymphomas under the WHO system

Case 10 : Benign mesothelial hyperplasia (reaction)

Cytology :

At low magnification : higly cellular specimen ; single cells to rare loose clusters ; one cell population with atypical -like cells ; rarety of organoid formations

At high magnification : moderately enlarged cells ; enlarged nuclei ; scant dense cytoplasm ; high N :C ratio ; rare multinucleated cells ; absence of true nuclear features of malignancy

Differential diagnosis : Malignant mesothelioma

Immunocytochemistry : is not useful for clear cut distinction between a benign mesothelial reaction and malignant mesothelioma

Antibody	Benign mesothelial reaction	Malignant mesothelioma
Keratin AE1/AE3	+	+++
EMA	+/-	+++
P53	+/-	+++
Desmin	++	+/-
BAP1	++	+/- (50% des cas)

- Strong EMA reaction has never be observed in a high proportion of benign mesothelial cell or mesothelial cell clusters. For some authors EMA is of value in distinguishing between benign reaction and malignant mesothelioma. Most mesotheliomas (98%) give a strong, often thick membrane reaction with EMA on most single cells and most cell clusters.

- The tumor suppressor gene BRCA1 associated protein 1 (BAP1) is involved in several cancers, including MMD. Loss of BAP1 expression is correlated with BAP1 somatic or constitutional genetic defects. Loss of BAP1 expression is an indicator of MMD in a context of mesothelial proliferation. The specificity of BAP1 was 100% on cytological and biopsy specimen for the diagnosis of malignancy in case of mesothelial proliferation.This immunohistochemistry could be integrated in the panel of immunostaining used for MMD diagnosis, either on histological or cytological samples.

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INVITED LECTURES

L 23

IMMUNOHISTOCHEMICAL PANEL AND DIFFERENTIAL DIAGNOSES IN PLEURA AND PERITONEUM.

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Tumours of the PLEURA are challenging for the pathologist especially on biopsy specimens. Different types of situations exist:

1/ Facing of a tumour with "epithelial features", the differential diagnosis is malignant mesothelioma vs metastatic adenocarcinoma. In this situation, immunohistochemical staining's are very helpful. Different panels are proposed in the literature with "mesothelial "markers and "carcinoma "markers (Henderson DW, 2013)

The most important point is that there is no specific marker to distinguish these 2 types of cells, so it is highly recommended to use panel with a broad spectrum cytokeratin antibody cocktail or a label for low molecular weight cytokeratin plus at least 2 "mesothelial" markers calretinin, WT1 such as plus at least 2 "carcinoma "markers TTF-1, BerEP4. (Henderson DW, 2013)

In case of an immunohistochemical pattern compatible with a mesothelial type of cell. The challenge is there to be certain that it is a mesothelioma vs benign proliferation. It is recommended that a diagnosis of mesothelioma always be based in IHC examination (Scherpereel A, 2010). Since recently no immunohistochemical marker was very useful in this field but now BAP-1 staining seems very promising (Churg A, 2016). BRCA-1 associated protein (BAP1) is a nuclear ubiquitin hydrolase that is believed to function as a tumour suppressor. It controls a number of functions like DNA repair and expression of genes related to cell cycle and cell proliferation. It can also induce cell death. BAP1 somatic mutations appear to be common in mesothelioma. The presence of biallelic mutations in BAP1 determined by molecular analysis correlates with loss of immunohistochemical staining. The epithelial mesotheliomas loose BAP1 more frequently than do mixed or sarcomatous forms. Stroma and inflammatory cells will always stain, thus providing a built-in control. The major drawback of BAP1 immunohistochemical staining relates to sensitivity, so that a mesothelial proliferation that retains BAP1 expression may still be malignant. The proposition of Churg A is that BAP1 test be run first, even in sarcomatous mesothelioma where BAP1 loss is less frequent, and if it is not lost, then p16 FISH should be tried. One important caveat must also keep in mind. Loss of BAP1 staining or deletion of p16 by FISH is not specific to mesotheliomas, but can be seen in a variety of malignancies. Thus, it is crucial to confirm, using established immunohistochemical stains, that the process in question is mesothelial before processing to BAP1 testing and/or p16 FISH (Churg A, 2016).

In case of carcinoma invading the pleura, the origin can be determined by IHC with a panel. (Husain AN, 2013) However there are no site-specific antibodies that are 100% specific and sensitive for metastatic carcinoma from most organs; therefore panels of antibodies are recommended. (Galateau-Salle, 2016). These include TTF-1 and Napsin A for adenocarcinoma of the lung, PAX-8 for renal and thyroid carcinoma, prostate-specific antigen (PSA-) and prostate-specific membrane antigen (PSMA) for adenocarcinoma of the prostate, CDX2 and cytokeratin 20 for adenocarcinoma of the gastrointestinal tract. Several markers for carcinoma of the breast including oestrogen receptor, progesterone receptor, gross cystic fluid protein (GCDFP-15), and mammaglobin. It should be noted that a relatively new marker for breast and bladder cancers, GATA-3, is expressed in more than half of mesothelioma and therefore has limited value or no use in this regard.

Other epithelioid neoplasms are present in the pleura(but these tumours usually don't express cytokeratin): large cell lymphoma (useful hematopoietic marker: CD45, CD20), malignant melanoma (useful: HMB-45, Melan A, SOX10), epithelioid hemangioendothelioma and epithelioid angiosarcoma. As precaution, it should be noted

that epithelioid hemangioendothelioma and epithelioid angiosarcoma may show some staining for cytokeratin although it is usually focal and these tumours express endothelial markers such as CD31, CD34, ERG and Fli-1. (Galateau-Salle, 2016)

2/ Facing of a tumour with sarcomatoid features. The difficulties are is it benign or malignant and mesothelioma vs sarcoma. Immunohistochemistry has a more limited role in the separation of sarcomatoid malignant mesothelioma from other sarcomas and sarcomatoid malignancies that involve the pleura. The vast majorities of sarcomatoid mesotheliomas stain positive for broad spectrum anticytokeratin antibodies, whereas most soft tissue sarcomas do not. (Galateau-Salle, 2016). Keratin stains can be negative in approximately 5% of sarcomatoid mesotheliomas. Calretinin is seen in only about 30% of cases. Mesothelial markers useful for the diagnosis of epithelioid mesothelioma are rather insensitive for sarcomatoid mesothelioma. Sarcomatoid mesothelioma may also show positivity for S-100, actin, or desmin, but these markers have no diagnostic specificity. One must be particular careful to distinguish sarcomatoid mesothelioma from other sarcomatoid malignancies involving the pleura that may be positive for cytokeratins. Monophasic synovial sarcomas may be identified by demonstrating the SY-SSX fusion protein by FISH. Sarcomatoid carcinoma may be positive for TTF-1, Napsin A or p40. Another differential diagnosis is solitary (localized) fibrous tumor. The latter stain positively for CD34 and BCL-2, and are usually keratin-negative. STAT6 is usually positive in SFTs and negative in malignant mesothelioma (Galateau-Salle, 2016)

In addition immunostaining for broad spectrum cytokeratins may be helpful in the diagnosis of desmoplastic malignant mesothelioma vs fibrous pleuritis, especially for the identification of invasion of adipose tissue or lung parenchyma by keratin-positive spindle cells. . (Galateau-Salle, 2016)

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The PERITONEUM must in all respects be considered an organ. This because of its anatomy (a continuous translucent membrane lining the abdominal cavity and covering the viscera), its physiology (supporting viscera, allowing free slipping of viscera, providing a pathway for blood vessels, lymphatics and nerves to the viscera, sealing off intraperitoneal infection), and its function as energetic deposit storing large amounts of fat. As for all organs the peritoneum is site of an absolutely peculiar and multiform pathology, ranging from reactive lesions to benign and malignant neoplasms. The 2016 BWP presentation will focus on low- and high grade malignancies of the mesoderm-derived mesothelium.

Peritoneal malignant mesothelioma (peritMM) is a rather rare disease accounting for approximately 400 new cases annually (10 to 15% of all mesothelioma cases) in the United States (Teta, 2008). PeritMM has a female predominance (Rodriguez, 2009), and childhood ALK-related cases have been reported (Loharamtaweethong, 2016). In women the link with asbestos exposure is less strong than it is for pleural MM. In men peritMM predominates over pleural MM after higher cumulative asbestos dose (Hodgson, 2000). Amosite rather than chrysotile is implicated in peritMM carcinogenesis. Other risk factors are radiation therapy, exposure to other mineral fibers like the silicate fiber erionite, SV40 virus infection and chronic peritonitis.

The 3 most-common molecular alterations in MM are loss of cyclin-dependent kinase inhibitor 2A (CDKN2A, p16), BRCA1-associated protein-1 (BAP1) and neurofibromin 2 (merlin or NF2) (Alakus, 2015 – Singhi, 2016). Loss of 16 by FISH occurs in approximately 25% of periMM. On the other hand, retention of p16 by immunohistochemistry is a useful prognostic indicator in epithelioid peritMM, with significantly prolonged survival in that group (Borczuk, 2005). The sensitivity for loss of nuclear expression of BAP1 in peritMM is 79%. Loss of the tumour suppressor gene NF2 has been found (together with LATS1/2 alterations) in MM by NGS-technology (Miyanaga, 2015 – Guo, 2015). The incidence of NF2 mutations in MM is estimated at 35%-50%. Singhi et al. studied the prognostic significance of NF2 in peritMM, and showed that hemizygous loss of NF2 by FISH is associated with an inferior prognosis (Singhi, 2016). In an attempt to exploit NF2 (and LATS1/2) diagnostically, Brandon et al. stained TMAs of benign mesothelial

proliferations and MMs, including 2 peritMMs with available comprehensive sequencing, using the Sigma-Aldrich HPA003097 antibody. The 2 peritMMs with frameshift and nonsense mutations retained NF2 immunreactivity, suggesting that, at least using the Sigma antibody, NF2 immunohistochemistry is not helpful for the diagnosis of peritMM (Brandon, 2016). Overall, in pleural and peritMM, NF2 immunohistochemistry had a sensitivity of 4% (1/25) and a specificity of 100% (0/43).

Alternative lengthening of telomere (ALT) mechanisms, causing telomerase-independent immortalisation – another hallmark of tumor cells - were found in 18% of peritMM (Villa Motta, 2008). Promoter mutations in TERT (the telomerase reverse transcriptase), leading to TERT overexpression, have not been searched for yet in peritMM.

For reporting peritMM 8 required elements and 7 recommended elements were agreed upon by the International Collaboration on Cancer Reporting (ICCR) Expert Panel (Churg, 2016). The required elements are: operative procedure, specimens submitted, macroscopic tumor site, histologic tumor type, margin status, extent of invasion, lymph node status and pathologic staging. Recommended elements are: clinical history, neoadjuvant therapy, tumor size, block identification key, mitotic count, response to neoadjuvant therapy, coexistent pathology, ancillary studies.

Well-differentiated papillary peritoneal mesothelioma (WD ppM) and multicystic peritoneal mesothelioma (MC peritM) are 2 rare variants of peritM, not related to asbestos exposure, occurring predominantly in young and middle-aged women, characterized by indolent behaviour and the potential for malignant transformation to peritMM. WD ppM is a small (<2 cm) typically incidentially found lesion. Differentiation from ordinary peritMM with focal papillary architecture is based on the lack of invasion, well-developed papillary architecture and lack of anisocytosis and anisonucleosis of the flat or cuboidal mesothelium lining the papilla. WD ppM is a low-grade malignancy cured by complete surgical resection (Hoekstra 2005). MC peritM is a large (10-15cm) tumor consisting of multiple grape-like clusters of mesothelium-lined cysts. Many women have a history of prior pelvic surgery, endometriosis or pelvic inflammatory disease. Recurrence rates are high (up 50%) after surgical treatment. The main differential diagnosis is with cystic lymphangioma, mesenteric/omental cysts, cystic epithelial neoplasms of the ovaries, endometriosis, peritMM, and pseudomyxoma peritonei (Weiss, 1988).

Concerning therapeutic options, the 9th International Congress on Peritoneal Surface Malignancies (PSM) was held in Amsterdam from 9-11 October 2014. The key messages of the Congress were that i) maintaining the patients' quality of life, patients with PSM should not undergo cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) if they are not expected to fully recover from the procedure, based on the preoperative diagnostic work-out, ii) CRS and HIPEC should be considered as a standard of care in pseudomyxoma peritonei and appendiceal tumors with peritoneal dissemination, iii) CRS and HIPEC should be the mainstay of treatment in patients with peritoneal metastases from colorectal cancer or peritoneal mesothelioma, iv) additional evidence from ongoing trials is required to further specify the exact indications in patients with advanced peritoneal metastases from ovarian cancer or from a primary gastric cancer (Seretis, 2015). The Peritoneal Surface Oncology Group International (PSOGI), in collaboration with the European Society of Surgical Oncology (ESSO), also launched an international surgical training programme for future peritoneal surface oncology surgeons, in order to obtain knowledge and skills to achieve a complete cytoreduction and be capable of administering the perioperative chemotherapy with safety, demonstrating acceptable morbidity and mortality rates. The outcomes of ongoing CRS and HIPEC trials will be discussed during the 10th International Congress of Peritoneal Surface Malignancies in Washington DC, from 17-19 November 2016 (www.psogi2016.com). Also, in 2016 the Journal of Peritoneum (and other serosal surfaces) (http://jperitoneum.org/) was born as a platform to report studies and ideas concerning oncology, inflammatory diseases and adhesions, septic diseases, gynecological diseases and endometriosis of organ peritoneum.

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L 24

MOLECULAR ASPECTS OF MESOTHELIOMAS: NGS AND FISH.

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Malignant mesotheliomas (MMs) are rare yet aggressive tumors for which both diagnosis and treatment continues to be challenging. The potential diagnostic, predictive or prognostic implications of molecular alterations in MMs were recently investigated.

The first studies reporting on the genomic background of MM identified recurrent alterations in a number of tumor suppressor genes. The most common genetic alterations are the inactivation of the tumor suppressor gene *NF2*; deletion of the 9p21 locus within a cluster of genes that includes *CDKN2A*, *CDKN2B* and *MTAP*; and mutation of *BAP1*. More recently, next-generation sequencing strategies have been used and have provided a more genome-wide view on the genetic landscape of MM. Doing so, rearrangements, point mutations and differentially expressed alternatively spliced genes have been reported. At this moment, there are no indications for the presence of a specific mutation in a single common driver gene, and hence the genetic cause for MM is thought to be more heterogeneous. In case of pleural MMs, the observed alterations seem to cluster in the tumor protein p53/DNA repair, cell cycle, mitogen-activated protein kinase, PI3K/AKT, Hippo and histone methylation pathways. As each of these pathways is important during tumor development, they provide interesting candidates for targeting with novel drugs.

For current diagnostic applications, p16/CDKN2A fluorescence in situ hybridization (FISH) and BAP1 immunohistochemistry still remain the most useful potential genetic markers. The p16/CDKN2A FISH assay using a commercially available dual-color FISH probe (Abbott Molecular) can be reliably performed on archival paraffin-embedded tissue (biopsy or cytology). p16/CDKN2A deletions are reported in up to 70% of primary epithelioid and 90% to 100% of sarcomatoid pleural MMs. The presence of this homozygous deletion is the best marker of malignancy in a mesothelial lesion, since it has not been reported so far in any of the benign lesions. However, absence of p16/CDKN2A loss by FISH therefore does not exclude MMs. The diagnostic finding of p16/CDKN2A loss is homozygous deletion rather than heterozygous. It should be reported that p16/CDKN2A deletion was observed in only 25% of peritoneal MMs. Moreover, p16 immunohistochemistry does not give the same results and cannot be substituted for p16/CDKN2A FISH. Of note, some studies have also reported that loss of p16/CDKN2A is associated with a poor prognosis.

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INVITED LECTURES

L 25

MUCINOUS AND PSEUDOMUCINOUS LESIONS OF THE PERITONEUM.

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Pseudomyxoma Peritonei

Pseudomyxoma peritonei (PMP) refers to the accumulation of mucin within the peritoneal cavity due to peritoneal spread of a mucinous neoplasm. Spread of mucinous neoplastic epithelium to the peritoneal cavity occurs most often with LAMN and mucinous adenocarcinomas of the appendix, but also of the colon, ovary, gallbladder, pancreas, and urachus. Peritoneal mucinous deposits tend to accumulate in particular areas, such as the greater omentum, the undersurface of the right hemidiaphragm, pelvis, right retrohepatic space, left abdominal gutter, and the ligament of Treitz. This so-called "redistribution" phenomenon is due to tumor accumulating at anatomic locations where ascitic fluid is resorbed from the abdomen, and in dependent areas in the abdomen where pooling of fluid occurs.

Pathology, Nomenclature, and Outcome

In 1995, Ronnett et al. separated peritoneal mucinous tumors into 2 categories based on cytologic and architectural features.[1] They proposed the term "disseminated peritoneal adenomucinosis" (DPAM) for "non-invasive" mucinous implants derived from an appendiceal "adenoma" (presumably LAMN) that contained scant strips of non-stratified mucinous epithelium with minimal to moderate atypia, at most focal tufting, and no significant mitotic activity. Peritoneal tumors characterized by more abundant epithelium arranged as glands, nests, or individual cells and with marked cytologic atypia were classified as "peritoneal mucinous carcinomatosis" (PMCA). These peritoneal tumors were derived from an appendiceal or intestinal mucinous adenocarcinoma.[2] Five and 10 year survival rates were 75% and 68% for patients with DPAM, and 14% and 3% for patients with PMCA.

In a study of 101 patients with pseudomyxoma peritonei by Bradley et al. found that patients with DPAM and intermediate grade peritoneal mucinous tumors had a similar outcome, and that they had a better prognosis than high grade peritoneal tumors.[3] They argued that DPAM is equivalent to well-differentiated adenocarcinoma. They proposed malignant terminology to describe PMP: mucinous carcinoma peritonei, low grade, or mucinous carcinoma peritonei, high grade. This two-tier system of grading is the standard in the current AJCC staging system.[4]

More recently, there has been increasing attention to the significance of signet ring cells. Shetty et al.[5] found that signet ring cells conferred a worse prognosis regardless of the percentage of the tumor they comprised. They proposed a three-tier grading system:

Grade 1: Peritoneal lesions with columnar, non-stratified epithelium without dysplasia or atypia and with abundant extracellular mucin (equivalent to DPAM).

Grade 2: Peritoneal lesions composed of extracellular mucin and cytological atypia (focal or widespread).

Grade 3: Peritoneal tumors with a signet ring cell component, regardless of the amount.

Other studies have confirmed that signet ring cells confers a worse prognosis, but there are still unresolved issues. For example, Shetty et al. suggested that signet ring cells can "hide" in low grade histology by mimicking macrophages. However, in a subsequent study,[6] the authors found that high grade tumors without signet ring cells had a similar prognosis to tumors with signet ring cells within mucin pools, and only the presence of signet ring cells invading tissue conferred a worse prognosis. Another issue that has not been addressed rigorously is the presence of degenerate tumor cells in mucin that are easily misinterpreted as signet ring cells ("pseudosignet ring cells").

In a recent report on 151 patients, Davison et al.[7] proposed a 3-tier grading system. They proposed

that tumors be scored according to the following features:

Cytologic grade: Low vs. High
Tumor cellularity: Low vs. High
Destructive invasion: Absent vs. Present
Presence of signet ring cells: Absent or Present
Angiolymphatic invasion: Absent or present
Perineural invasion: Absent or present

The definitions of these parameters were as follows:

1. Cytologic grade

Low grade: flat strips of cells with mildly enlarged, hyperchromatic nuclei with nuclear stratification, and maintenance of cell polarity without significant mitotic activity or prominent nucleoli.

High: Enlarged, vesicular nuclei with full-thickness stratification, loss of nuclear polarity, prominent nucleoli, cribriform or micropapillary growth, and increased mitotic figures. These changes had to occupy greater than 10% of the tumor.

2. Tumor cellularity: Based on the overall percent of tumor that contained neoplastic epithelium by visual estimation of the entire case

   Low cellularity: < 20%

   High cellularity: > 20%

3. Destructive invasion: Unequivocal infiltration into subjacent normal tissues with the following patterns: 1) infiltrating, haphazard, irregular, jagged neoplastic glands or single cells associated with desmoplastic stromal reaction, 2. expansile and confluent cribriform glandular growth, or 3. small nests, glands, or single neoplastic cells floating within small pools of mucin with or without desmoplastic stromal reaction.

Peritoneal tumors that had at least one of these parameters were classified as high grade (grade 2), except for signet ring cells, which qualified as grade 3. Destructive invasion or cytologic grade was the most common adverse histologic features. The small mucin pool pattern was the most common pattern of invasion.

Efforts to achieve consensus: The Peritoneal Surface Oncology Group International project

In 2013, experts in PMP, through the sponsorship of the Peritoneal Surface Oncology Group International (PSOGI), agreed to participate in a modified Delphi process to arrive at consensus terminology for PMP and mucinous appendiceal tumors. Surveys were disseminated via email, and the answers were collated and sent back to the participants. This was followed by additional rounds of questions. Agreement and consensus were defined based on the number of respondents and the degree of contention for the question. The final consensus document was published in January, 2016.[8] As regards PMP, the group arrived at the following consensus statements:

1. Pseudomyxoma peritonei is the intraperitoneal accumulation of mucus due to mucinous neoplasia characterized by the redistribution phenomenon. It can include mucinous ascites, peritoneal implants, omental cake, and ovarian involvement. It most commonly arises from appendiceal neoplasia.
2. PMP should be considered a malignant condition.
3. The grade of PMP (and not the primary tumor) should be the parameter that distinguishes anatomic stage IVA disease from IVB.
4. Lymph node involvement should not be classified as high grade.
5. The classification of PMP based on grade would be as follows:

Lesion	Terminology
1. Mucin without epithelial cells	Acellular mucin
2. PMP with low-grade histologic features	Low-grade mucinous carcinoma peritonei OR Disseminated Peritoneal Adenomucinosis (DPAM)
3. PMP with high-grade histologic features	High-grade mucinous carcinoma peritonei OR Peritoneal mucinous carcinomatosis (PMCA)
4. PMP with signet ring cells	High-grade mucinous carcinoma peritonei with signet ring cells OR Peritoneal mucinous carcinomatosis with signet ring cells

INVITED LECTURES

Reporting of peritoneal mucinous neoplasia

When reporting mucinous tumors of the peritoneum, the most important factors are:

1. The presence or absence of mucinous epithelial cells.
2. The grade of the tumor. In the current AJCC, a two-tier system for mucinous neoplasia is advocates. However, a three-tier system modeled after Davison et al. is expected to be advocated in the new AJCC system.
3. Recognizing that diligent sampling is required to exclude epithelium in peritoneal mucin because this drives prognosis more so than the primary tumor. Keratin stains are not necessary and may be confusing due to mesothelial cells. CDX2 stains may be more useful, but still largely unnecessary in most cases.
4. Acellular mucin within the peritoneal cavity will be pM1a in the new AJCC system.

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THE BELGIAN MESOTHELIOMA REGISTRY IS A VALUABLE DATA SOURCE FOR POPULATION-BASED RESEARCH ON MALIGNANT MESOTHELIOMA.

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1. 30 Years Belgian Mesothelioma Registry: a valuable data source

Malignant mesothelioma (MM, ICD10:C45) is a rare but aggressive cancer with a latency period up to several decades. Most commonly originating from the pleura, MM is a disease for which exposure to asbestos is a well-documented etiological factor. The diagnosis of MM remains in some cases difficult: the high degree of morphologic heterogeneity can be mimicked by numerous secondary tumours, resulting sometimes in uncertain diagnoses. Depending on clinical circumstances it can also be difficult to obtain adequate and/or sufficient biopsy material for histological analysis in order to make a firm diagnosis.

In 2006, the Asbestfonds/Fonds Amiante (AFA) was established, aiming to certify diseases caused by exposure to asbestos in Belgium and to provide financial compensation. The diagnosis of mesothelioma is confirmed by a Certifying Committee, i.e. the Belgian Mesothelioma Commission composed of an expert panel of pathologists meeting monthly. The data of all revised diagnoses are collected in the Belgian Mesothelioma Registry (BMR).

The BMR started in 1986 with 6 mesothelioma cases per year. At that time, meetings providing second opinion were held 4 times in a year. Since 2000 the expert panel meets monthly, with more than 250 cases of MM diagnoses a year. Cases are sent by the AFA for certification of the MM diagnosis and by pathologists for second opinion.

The BMR diagnostic immunopanel contains markers for mesothelial cells (calretinin, Wilms tumor protein [WT1], cytokeratin [CK] 5/6) and for non-mesothelial cells (epithelial membrane antigen [EMA], polyclonal carcinoembryonic antigen [CEA-pol], thyroid transcription factor 1 [TTF-1]) with CK AE1-AE3 as an extremely useful marker for evaluating the invasion pattern and the depth of invasion. During the different years, the choice of diagnostic immunopanel changed and other markers were added: paired box gene 8 [PAX8], BRCA1 associated protein-1 [BAP1] and recently p16 fluorescence in situ hybridization [p16 FISH]. The diagnosis is made according a probability score ranging from A to F (A: certain MM, F: MM excluded).

Referral cases containing bulky tumor masses rarely pose diagnostic problems, small size biopsies however do often because of the lack of immunohistochemistry (IHC) after exhaustion of the sample.

As clinical skills detecting early cases evolve, the diagnostic challenge for pathologists changes in parallel. Very early mesothelioma cases with minimal invasion or mesothelioma in-situ (atypical mesothelial hyperplasia) are more often diagnosed (in BMR files 0.76%-1.31%). The use of BAP1 IHC and p16 FISH are welcome adjuncts as diagnostic tools in this matter.

According to the BMR files, MM involves most frequently in the parietal pleura, visceral pleura and peritoneum. Locations at pericardium and tunica vaginalis remain rare.

Epithelioid mesothelioma represents 73.6% of all diagnosed cases, followed by 13.7% of sarcomatoid, 9.9% of biphasic and 1.4% of desmoplastic subtypes.

In the BMR, metastatic invasion is observed by primary tumors from the lung, kidney, ovary, breast, colon and from urothelial origin.

Particularly difficult is the differential diagnosis from metastatic invasion of breast carcinoma.

Immunomarkers overlap considerably between both diagnoses as primary mesothelioma of the pleura cases, developed after prior irradiation of the thoracic wall, are registered (N=4). All cases of MM in women are stained for PAX8 to exclude metastatic invasion by an ovarian neoplasm.

In spite of 30 years' experience of MM diagnostics, the panel members still consider any presented case as challenging and requiring the highest level of attention to details such as growth pattern, nuclear pleomorphism and immunohistochemical reaction of the neoplastic cells.

2. Research example: Assessing completeness and correctness of malignant mesothelioma registration at the BMR and the Belgian Cancer Registry

Background

Next to the BMR, the Belgian Cancer Registry (BCR) is another population-based registry for MM (besides other cancer types). The BCR can be considered almost complete for incidences from 2004 onwards. Cancer registration has a firm legal basis, as defined in different laws[1,2]. The data flow relies on all information (notifications) coming from the oncological care programs (clinical network) and the laboratories for pathological anatomy (pathological network).

As both the BMR and BCR are collecting information regarding MM for the Belgian population, comparing these databases might deliver insights on completeness and correctness of MM registration by both instances.

Objectives

To assess and enforce completeness and correctness of MM registration at both the BCR and BMR, the current study aimed to compare information from three independent databases, i.e. the BCR standard cancer registration, the BMR and the population-based mortality statistics (death certificates (COD)).

Given a median overall survival of 10.7 months[3], a secondary goal was to provide more insights in patient and tumour characteristics of MM long-term survivors, suspicious for wrong diagnoses.

Material and Methods

The study cohort (N=2,388) included all cases of MM and malignant neoplasms of pleura (MNP; ICD10:C38.4) reported to the BCR (incidence years 2004-2012; N=2,343), all patients reviewed by the pathology commission of the BMR (2004-2012; N=2,019), and COD data for all Belgian citizens (2004-2013).

Effort was put in making the BMR electronically available. This database contains different variables describing patient data, applicant data, the date of the meeting of the mesothelioma commission, and tumour information (diagnosis, certainty of diagnosis, sample type, immunohistochemical (IHC) markers with result).

An internal registration project was set up by the BCR aiming to catch additional information found in the pathology protocols (APD) provided by the pathological network, with a particular focus on diagnosis and performed IHC tests. Pathology protocols were available in 2,059 cases (86.2%) in which information on performed IHC staining was found in 1,740 cases (84.5%).

All available data were compared for diagnosis and performed IHC tests as derived from the available pathology reports at the BCR or registered in the BMR.

Using the vital status as obtained from the Crossroads Bank for Social Security (CBSS) based on the patient's unique national security number, it was possible to identify long-term survivors, defined as patients that survived more than 3 years after their diagnosis.

For COD data, a probabilistic coupling, based on the niscode (numeric code for regional areas in Belgium) of the residence at time of death, the date of birth, the date of death and gender, of these data with the standard cancer registration database resulted in a linkage of 2,086 cases out of 2,126 deceased between 2004-2013 (98.1%).

Results

Before the start of this project, the BCR counted 2,343 cases of MM (N=2,291) and MNP (N=52) between 2004 and 2012 mainly diagnosed in men (male/female ratio=4:1) with a median age

of 71 years at time of diagnosis. Malignant pleural mesothelioma represented 92% of all MM cases. Over this period, no significant time trend was observed in MM incidence (AAPC=-0.8, CI=[-2.4;0.8]).

Of the 1,583 MM cases present in the BMR, 1,538 (97.2%) were identified in the BCR database with a concordant MM diagnosis. Vice versa, 1,635 (69.8%) of all MM cases present in the BCR were found in the BMR database, for which a concordant diagnosis was made in 94.1%. For the remaining 97 patients, the BMR expert panel concluded either in benign disease (N=29) or a secondary tumour (N=41) to mimic MM, or could not provide a firm diagnosis (N=27). 656 (28.6%) MM cases registered at the BCR, could not be identified in the BMR database. Some of these cases might have been discussed by Mesothelioma Commission only after 2012 and data was therefore not available. Of the 45 cases reported as MM by the BMR and not found in the BCR database, 9 patients were identified with a MM diagnosis before 2004 registered by the BCR. Regarding the remaining 36 patients, 10 where diagnosed with lung cancer (ICD10:C34) and 10 with a malignant neoplasm without specification of site (ICD10:C80).

The combination of information originating from these two databases permitted to decrease the percentage of MM cases originally registered with unspecified morphology in the BCR database, from 25.8% to less than 1%. For 116 cases, the specified morphology diverged between the BCR and BMR and need to be reviewed. Small amounts of tissue samples available for analysis in combination with tumour heterogeneity might explain most of these discordances.

Results from IHC tests, present in either the BMR and/or APD, were available for 95.9% (N=1,475) of concordant MM cases: the most performed IHC stainings were calretinin (95.6%), CEA-pol (76.7%), CK5/6 (85.1%), EMA (81.8%) and TTF-1 (72.1%). Different IHC test patterns were observed in the tissue samples, in line with different MM histological subtypes. The APD/BMR comparison confirmed the difficulty to obtain a firm diagnosis for MM, and the need for expert pathologists in this matter. For some cases, it appeared that additional IHC tests were performed by the BMR, or that IHC stainings performed by the referring APD laboratory were reviewed by the expert panel of the BMR. For other cases, the referring APD laboratory received (sometimes years later) new tissue samples leading to a distinct diagnosis as the initial one, without providing this material to the Mesothelioma Commission.

For 20.9% (N=428) of MM cases registered at the BCR and linked with COD data (N=2,045), C45 was not mentioned as any cause of death. Vice versa, some patients found in BCR for which COD data mentioned C45 or C38.4 as cause of death, were registered at the BCR with a different diagnosis. This was the case for 150 MM and 158 MNP according to COD data. In both series, most of these cases appeared to be registered at BCR as C34 (malignant neoplasm of bronchus and lung: 42.7% of MM and 53.8% of MNP), or as C80 (21.3% of MM and 31.7% of MNP). For these discordant cases, additional information available in the pathology protocols present at BCR or in the BMR will be explored in order to obtain a firm diagnosis.

The COD data were used as a starting point to set up a trace-back system to recover mesothelioma cases that remained unknown to the BCR ("death certificate only" cases). In total, C45 was mentioned among the causes of death in COD data between 2004-2011 for 165 cases that remained unknown to BCR. The place of death as described in the COD data in broad categories (home, nursing home, hospital, other), in combination with the niscode of the place of death led to the identification of hospitals in which presumably patients with a mesothelioma died but for whom this diagnosis was not notified to the BCR. In a first phase, BCR has contacted two hospitals (one from the Flemish and one from the Walloon Region) to explore the feasibility of retrieving information on these patients.

Long-term survivors, defined as patients who survived at least 3 years after being diagnosed with MM, represented 11.1% (N=170) of concordant MM cases. This subpopulation was characterized by a higher percentage of peritoneal MM, a less pronounced male/female ratio, a higher predominance of the epithelioid subtype and a younger age profile.

Conclusions

Given the proven feasibility of comparing different population-based data sources, additional in-

depth analyses are planned in order to optimise correctness and completeness of MM registration in Belgium.

Discordant diagnoses will be explored in detail and if necessary, a pathology revision will be performed and/or sources will be contacted. Reasons for under-registration and discordances in both databases will be investigated. The percentage of diagnoses that changed from an initial MM diagnosis to another diagnosis and vice versa might also be determined.

For some cases not registered by the BMR, an underuse of specific immunohistochemical markers by certain pathology laboratories might be seen. In that situation, a revision of the cases by the Belgian Mesothelioma Commission could be envisioned.

In function of the results of the ongoing feasibility exercise, the trace-back system to recall eventually missing diagnoses based on the certificates of death, will be further extended.

Once agreement on diagnoses is achieved, special attention will be payed to long-term survivors: for those case for which the diagnosis of MM remains confirmed, the available data will enable further analyses including in-depth profiling of long-term survivors.

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BONE FORMING LESIONS.

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Although there are not so many entities within this group, the diagnosis of bone forming lesions can be challenging for pathologists. As holds true for any bone lesion, a diagnosis should never be made without correlation with the imaging features.

Osteoma

Osteomas are benign lesions that are more frequently solitary and sporadic than multiple and syndromatic (Gardner syndrome). They are mainly seen in flat bones, the craniofacial bones being most frequently involved. In the majority of cases they consist of compact hyperostotic bone, with inconspicuous osteoblasts (1). Osteomas are surface lesions, if an intramedullary focus of compact bone is seen, it concerns a bone island.

Osteoid osteoma

This tumor corresponds to 10-15 % of all benign bone tumors and is mainly seen in children and young adults. Osteoid osteomas are rare in patients younger than 5 years and older than 35 years. Any bone can be affected, but long bones of lower extremities are frequently and craniofacial bones are rarely involved. Osteoid osteomas typically show a targetoid appearance on imaging and are smaller than 2 cm. Pain, relieved by NSAIDs is very characteristic. On histology, the nidus should be present, consisting of irregular islands of partly mineralized osteoid trabeculae surrounded by osteoblasts and embedded in a highly cellular to vascular stroma. Hypervascular sclerotic bone surrounds the nidus. There are different treatment modalities: curettage, en bloc resection, ablation… The nidus should be removed or destroyed, and then recurrence is rare. The growth potential is very limited (2).

Osteoblastoma

Osteoblastoma is related to osteoid osteoma but is 5 times less common than osteoid osteoma (3). It occurs in the same age group as the latter. 40-55% of tumors affect the posterior elements (posterior arch) of spine and sacrum, the proximal/distal femur or proximal tibia, and they are not uncommon in craniofacial bones (mandible). The imaging is less specific, but often lytic, measuring 2-10 cm (<2cm=osteoid osteoma!). Pain is also often present, but with less response to NSAIDs. The histology is identical to osteoid osteoma: sharply demarcated haphazard network of osteoid and woven bone in a fibrovascular stroma. Osteoblastic rimming is prominent, mitoses can be seen but no atypical ones, cartilage is rare. Infiltration of host bone should not be seen, otherwise it concerns an osteosarcoma. The osteoblasts can become very plump with vesicular nuclei: epithelioid osteoblastoma. Formerly this was also called 'aggressive' osteoblastoma, but the prognosis is not different. If feasible, en bloc resection is advised, in the case of curettage recurrence is seen in+/- 20% of cases. Osteoblastomas have an unlimited growth potential, but malignant transformation is not convincingly documented (4).

Conventional osteosarcoma

This is the most common primary high grade bone sarcoma, it is twice as common as chondrosarcoma and three times as common as Ewing sarcoma. A sporadic setting is much more common than a syndromatic one (Li-Fraumeni (germline p53 mutation)), hereditary retinoblastoma syndrome (germline RB1 mutation)). There is a typical bimodal age distribution, 70% of cases occur in children between 10 and 20 years, 30% is seen in adults older than 40 years. In patients older than 60 yrs, half of the osteosarcomas are secondary to Paget's disease. Any bone can be involved, but 50% of cases involve the knee region (distal femur, proximal tibia), or the proximal humerus (metaphysis). In adults, tumors are more axially (pelvis, spine) located. Unfortunately there is very often an important patient and doctor's delay, and at diagnosis a painful

mass is often detected. Imaging is very variable but often a large, destructive, mixed lytic/blastic lesion, with extension into the soft tissue is found. Conventional osteosarcoma looks usually like a high grade sarcoma with spindled, round, epithelioid, or plasmacytoid cells with prominent atypia, numerous (atypical) mitoses++, and infiltrative growth. Neoplastic bone should be present, showing woven, disorganized (lace-like) deposition of bone, intimally associated with tumor cells.

Osteoblastic (50%), chondroblastic (25%, high grade malignant hyaline cartilage), or fibroblastic (25% herringbone, storiform spindle cells) can be discriminated, but this is of no importance, giant cells can be present as well (5). Immunohistochemistry is not useful, and even confusing (S100, keratin, EMA, CD99… can be positive). Thanks to chemotherapy the prognosis has increased considerably, and the histopathological evaluation of the resected tumor after chemotherapy differentiates between good (>90% necrosis) and bad (<90% necrosis) (6).

The vast majority of osteosarcomas correspond to the conventional type. There are, however, various subtypes, some of which are very rare (≤2% of osteosarcomas) and will not be discussed here.

Teleangiectatic osteosarcoma
Small cell osteosarcoma
Low grade central osteosarcoma
Parosteal osteosarcoma
Periosteal osteosarcoma
High grade surface osteosarcoma
Secondary osteosarcoma

All these tumors are high grade, except the low grade central and parosteal type.

Telangiectatic osteosarcoma

This variant represents 4%-10% of osteosarcomas and is most frequently seen in the 2nd decade. The metaphysis of the distal femur is most often involved, followed by the proximal tibia, the proximal humerus and the proximal femur. A large lytic lesion is seen on imaging, with extensive bone destruction and typical fluid/fluid levels like in an aneurysmal bone cyst. The histological diagnosis can be very challenging and correlation with imaging is of utmost importance. The tumor looks very aneurysmal bone cyst-like with blood-filled spaces delineated by septa with giant cells. In the septa pleomorphic, atypical cells should be looked for, and malignant osteoid is often very focal. Therapy and prognosis are as in the conventional type (7).

Parosteal osteosarcoma

4-5% of osteosarcoma corresponds to this type, in fact it is the most frequent surface osteosarcoma and is three times more common than periosteal osteosarcoma. The peak incidence is in the 3rd decade. Patients often complain of a painless swelling and flexion restriction due to a heavily mineralized mass attached to the cortex. 70% of these tumors is localized at the posterior surface of distal femur or tibia, the humerus is also rarely involved. On histology, parosteal osteosarcoma often looks benign and in the absence of imaging data the diagnosis can often not be made. Classically, fascicles of non-atypical spindle cells admixed with parallel bone trabeculae are seen, +/- osteoblastic rimming. About half of the cases contain cartilage islands, 50% transgress the cortex and invade the medullary cavity. In about 15-25% dedifferentiation to high grade (osteo)sarcoma can be found. Of major interest, MDM2 and CDK4 expression/amplification is present by immuno/FISH, which can be very useful diagnostically. Upon complete resection the 5 yr survival is 91%. Marrow invasion is of no influence on the prognosis. Chemotherapy is only administered if there is dedifferentiation (15-20%), then the prognosis as in the conventional type (8).

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VASCULAR AND NOTOCHORDAL LESIONS.

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Vascular tumours of bone are a heterogeneous group of lesions with variable prognosis. According to the WHO Classification they encompassed haemangiomas, epithelioid haemangiomas, epithelioid and pseudomyogenic haemangioendotheliomas and angiosarcomas. Bone haemangiomas are frequent incidental findings especially in vertebrae with typical radiological features. They are histologically similar to their soft tissue counterparts. Pseudomyogenic haemangiothelioma (epithelioid sarcoma-like) is a rare recently described soft tissue tumour of intermediate malignancy affecting children and young adults. The tumour is usually situated in limbs and involves skin, muscle and bone. The tumour is composed of sheets or fascicules of plump spindle cells with abundant brightly eosinophilic cytoplasm with usually prominent stromal neutrophils. Tumour cells show diffuse expression of Keratin and ERG. Most are positive for CD31 while CD34 is negative. Recently a *SERPINE1-FOSB* fusion gene was reported in these tumours.

"Epithelioid vascular tumours" correspond to epithelioid haemangiomas, epithelioid haemangioendotheliomas and angiosarcomas. They may have overlapping features. Recently molecular studies reported more or less specific abnormalities allowing to a better classification. *A ZFP36-FOSB* fusion gene was found in "atypical" epithelioid haemangiomas while epithelioid haemangioendotheliomas harbor *WWTR1-CAMTA1* or less frequently *YAP1-TFE3* gene fusion. In contrast no specific molecular alteration is find in angiosarcoma, a highly malignant tumour which has to be distinguished from a metastasis of a carcinoma when the morphology is epithelioid.

Notochordal tumours encompassed benign notochordal cell tumour and chordomas. They occur in the bones of the base of skull, the vertebral bodies of the mobile spine and the sacrococcygeal bones. Recently extraosseous cases of both tumours have been reported. Few extra-axial bone chordomas have also been reported. Most Benign notochordal cell tumours are incidental findings. They are well-limited tumours without lobular architecture or myxoid matrix. The tumour cells are vacuolated and mimic adipocytes. Bone trabeculae are sclerotic. In contrast chordomas are infiltrative and expansile tumours made of cords and ribbons of "physaliphorous cells"in a myxoid extracellular matrix. Atypias, mitoses and necrosis can be found. Chordoid chordoma also contains hyaline cartilaginous matrix. Dedifferentiated chordoma is a biphasic tumour with features of a chordoma juxtaposed to a high grade undifferentiated sarcoma. Notochordal tumours express keratins, EMA and S100 protein. Brachyury is thought to be a highly specific nuclear marker for notochordal tumours though it was recently reported that it is consistently expressed by in Germ cell tumors and small cell carcinomas.

INVITED LECTURES

L 30

CARTILAGINOUS TUMOURS.

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Chondrosarcoma is the second most frequent primary bone malignancy, predominantly affecting adults[1]. They usually arise from their benign precursor lesions: peripheral chondrosarcoma arising in osteochondroma (at the surface of bone, with EXT mutation) and central chondrosarcoma arising in enchondroma (in the medulla of bone, with IDH mutation). The prognosis is strongly correlated with histological grading. Grade I chondrosarcoma, now reclassified as an atypical cartilaginous tumor, is locally aggressive, but typically does not metastasize[1]. High grade chondrosarcomas (Grade II and III) have an increased metastasizing capacity alongside poor patient survival. Surgery is the mainstay of treatment. In the event of tumor location at a nonresectable site, such as in the skull or pelvis, or metastatic disease, there is still no curative treatment[2]. Chondrosarcoma is notorious for its primary resistance to conventional chemo- and radiotherapy[2,3].

For chondrosarcoma, a multistep genetic model has been devised: while specific mutations (IDH, EXT) cause the benign precursor lesions, high grade chondrosarcomas have complex karyotypes[4] and it is as yet unclear whether the original mutations are still driving tumor cell proliferation. IDH (isocitrate dehydrogenase) is involved in the tricarboxylic acid cycle, and the exact mechanism by which deficiencies in metabolic enzymes cause cancer is so far unknown. Recent evidence point to an important role for epigenetic changes [5-7]. Moreover, 96% of them contain alterations at some level in the pRb pathway or TP53 pathway[8,9]. The EXT genes encode type II transmembrane glycosyltransferases – exostosin 1 and exostosin 2 – that form a hetero-oligomeric complex in the Golgi apparatus catalysing the chain elongation of heparan sulphate [10-12], thereby orchestrating the diffusion, concentration and activation of various growth factors, signalling molecules and cytokines, including Indian Hedgehog (IHH). Benign osteochondromas can occur sporadically, or within the hereditary syndrome of multiple osteochondromas, in which patients carry germline mutations in EXT.

Both enchondroma and osteochondroma can progress to malignant chondrosarcoma. In both, the pRb and p53 pathway are presumed to play a crucial role [4,9]. Moreover, in high grade chondrosarcomas, the IDH mutation is no longer essential for tumor growth [13,14]. Moreover, whole genome sequencing studies have shown various inactivating alterations in the COL2A1 gene in chondrosarcomas [15,16]. In addition, we previously identified activating NRAS mutations in 12% of conventional central chondrosarcomas, especially those of high histological grade[17].

In addition to the conventional central and peripheral chondrosarcomas, rare but distinct subtypes are recognized, including periosteal, dedifferentiated, mesenchymal and clear cell chondrosarcoma.

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INVITED LECTURES

L 31

A PRACTICAL APPROACH TO SMALL BOWEL BIOPSY INTERPRETATION: COELIAC DISEASE AND ITS MIMICS.

R. Paï / Scottsdale, U.S.A.

Abstract:

While coeliac disease is the most common cause of villous remodeling and intraepithelial lymphocytosis in the proximal small bowel, there are many entities that can mimic its histologic appearance. The purpose of this presentation is to discuss normal small bowel histology and the differential diagnosis of coeliac disease. Approaches to evaluate increased intraepithelial lymphocytes are presented, followed by a detailed discussion of the pathology of coeliac disease. Particular emphasis is given to those conditions that cause intraepithelial lymphocytosis in the setting of preserved villous architecture, although other important entities, such as peptic injury, idiopathic inflammatory bowel disease, medication injury, eosinophilic (allergic) gastroenteritis, autoimmune enteropathy, common variable immunodeficiency, and infections are also discussed.

L 32

LYMPHOMATOID POLYPOID LESIONS OF THE DIGESTIVE TRACT.

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Lymphomatous polyposis of the gastrointestinal tract is considered to represent mantle cell lymphoma. However, less commonly, it is a mucosa-associated lymphoid tissue (MALT) lymphoma, a chronic lymphoid leukemia, a T-cell lymphoma or a plasmocytoma. Polyposis in the duodenum has been reported with follicular lymphoma. These subtypes cannot be clearly differentiated on morphology alone. The macroscopic appearance of polyps in lymphomatous polyposis is not specific underlying the value of pathology and especially immunohistochemistry. Gastro-intestinal symptoms are also non specific and they may be abdominal pain, diarrhea, obstruction, hematochezia, or less frequently protein-losing enteropathy, intestinal malabsorption, and acute abdomen due to perforation. As prognosis in mantle cell lymphoma is considered poor, differential diagnosis is crucial from a chronic lymphoid leukemia (CD5+, CD23+), a follicular lymphoma (CD10+, Bcl6+, CD5–, CD 23–), and a MALT lymphoma (CD138 +/–, CD10–, Bcl6–, CD5–, CD 23–). Gastro-intestinal biopsies with histologic examination, immunohistochemical staining and molecular biology analysis are the gold standard for a proper diagnosis.

Our review emphasizes the importance of differential diagnosis of lymphomatous polyposis in evaluating prognosis and the most suitable therapeutic regimen.

L 33

TUMOUR REGRESSION GRADING IN DIGESTIVE TUMOURS.

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Abstract

Multimodal therapy concepts have been successfully implemented in the treatment of locally advanced gastrointestinal malignancies. The effects of neoadjuvant chemo- or radiochemotherapy such as scarry fibrosis or resorbtive changes and inflammation can be determined by histopathological investigation of the subsequent resection specimen.

Several Tumor regression grading (TRG) systems exist, which aim to categorize the amount of regressive changes after cytotoxic treatment mostly refer onto the amount of therapy induced fibrosis in relation to residual tumor or the estimated percentage of residual tumor in relation to the previous tumor site. TRG is an important prognostic factor in locally advanced gastrointestinal carcinoma following neoadjuvant treatment, since complete or subtotal tumor regression has shown to be associated with better patient´s outcome. The prognostic value of TRG may even exceed those of currently used staging systems (e.g. TNM staging, in particular ypT category).

Characteristic histomorphologic features of tumor regression will be presented. Commonly used TRGs for upper gastrointestinal carcinomas such as the Mandard grading and the Becker grading system for upper gastrointestinal carcinomas and the Dworak grading system and the AJCC for rectal cancer system will be introduced and discussed. Some site specific issues of TRG in different tumors will be covered. A proposal of macroscopic and histopathologic work up and standardized reporting of TRG will also be included.

Handout

The following handout is modified and updated from:

Thies and Langer, Tumor Regression Grading of Gastrointestinal Carcinomas after Neoadjuvant Treatment, Front Oncol 2013 [1]

Introduction

Preoperative chemo- or radiochemotherapy followed by surgery or perioperative treatment currently represents the standard approach for locally advanced esophageal, gastric and rectal carcinomas, providing survival benefit for the patients compared to surgery alone. In particular, patients with complete or subtotal regression of the tumor show significant improved survival rates[2-5]. The effects of neoadjuvant chemo- or radiochemotherapy can be determined by histopathological investigation of the posttherapeutic resection specimens[6-8]. Several tumor regression grading (TRG) systems exist, which aim to categorize the amount of regressive changes after cytotoxic treatment. They refer to the amount of therapy induced fibrosis in relation to residual tumor[9, 10] or the estimated percentage of residual tumor in relation to the previous tumor site[7, 11]. Due to their prognostic impact they may represent a morphological marker for subsequent guiding of patients after neoadjuvant treatment and surgery.

Regressive alterations of the tumors after neoadjuvant therapy.

Macroscopy can already roughly estimate the degree of tumor regression, however, fibrosis may resemble vital tumor so that careful histomorphologic investigation is mandatory. The histologic appearance of regression basically represents a subacute-subchronic inflammation because most tumors are resected after a delay of several weeks after completion of the preoperative treatment.

Significant regressive changes may result in complete disappearance of malignant cells and replacement of the tumor by fibrous or fibro-inflammatory granulation tissue. Signs of resorbtion, like histiocytic reaction with hemosiderin-laden and foamy macrophages, cholesterol deposits and foreign body reaction, as well as dystrophic calcifications can be seen[6, 7, 12, 13]. A finding of adenocarcinomas treated by neoadjuvant therapy is the presence of acellular mucin lakes[7, 14]. They should not be considered as viable residual tumor, but warrant an intense search for residual vital tumor cells[12]. On the cellular level, both the residual malignant cells, and non neoplastic tissue can show eosinophilic cytoplasm, vacuolization of cytoplasma or undergo oncocytic differentiation. Marked nuclear atypia with hyperchromasia, karyorrhexis, pyknosis, or enlargement of nuclei with sometimes bizarre formations are frequent findings. Giant cells may also be present. Mitoses are found rarely in contrast to apoptotic figures. [7, 12, 13].

Classification of tumor regression

Tumor regression grading (TRG) systems aim to categorize the amount of regressive changes after cytotoxic treatment in order to demonstrate potential prognostic information based on objectively determinable histopathologic findings. The tumor regression grading systems according to Mandard[10], Becker[7], Dworak[9] or the AJCC[15] are examples for commonly used TRGs.

Mandard Tumor Regression Grading System

TRG	Criteria
1	complete regression (= fibrosis without detectable tissue of tumor)
2	fibrosis with scattered tumor cells
3	fibrosis and tumor cells with preponderance of fibrosis
4	fibrosis and tumor cells with preponderance of tumor cells
5	tissue of tumor without changes of regression

Becker Tumor Regression Grading System

TRG	Criteria
1a	no residual tumor/tumor bed + chemotherapy effect
1b	< 10% residual tumor/tumor bed + chemotherapy effect
2	10-50% residual tumor/tumor bed + chemotherapy effect
3	> 50% residual tumor/tumor bed + chemotherapy effect

Dworak Tumor Regression Grading System

TRG	Criteria
0	no regression
1	predominantly tumor with significant fibrosis and/or vasculopathy
2	predominantly fibrosis with scattered tumor cells (slightly to recognize)
3	Only scattered tumor cells within fibrosis with/without acellular mucin
4	no vital tumor cells detectable

AJCC Tumor Regression Grading System

TRG	Criteria
0	no residual tumor cells;
1	single cells or small groups of cells
2	residual cancer with desmoplastic response
3	minimal evidence of tumor response

Prognostic significance of TRG

Numerous studies show the prognostic relevance of TRGs. For upper gastrointestinal cancers there is the strongest evidence for the association between TRG and patient outcome. There is the general observation that patients with complete tumor regression do best and that cases with >50% tumor/predominant tumor mass do worst. For "subtotal" or "partial" regression data are still conflicting For rectal cancer, works have also provided convincing evidence to support TRG as predictor of survival. TRG, especially in terms of complete regression, therefore is considered to representing a potential tool to guide therapy in patients with rectal cancer as well [16, 17].

Critical issues of TRG

The most critical issues are inter- and intra-observer variability and the lack of standardization [18] Some studies, however, demonstrate a good reproducibility of TRGs; they show that the most frequent source of disagreement was assessment of the relative amount of fibrosis, while displacement of epithelium or the misinterpretation of acellular mucin was a minor source of disagreement [19]. Studies comparing the "% approach" showed a slight advantage of this system over the "fibrosis approach" in terms of interobserver agreement and prognostication [20, 21]

Standardization of work up and reporting TRG

A crucial issue in surgical pathology of resection specimen of tumors treated by neoadjuvant therapy is the difficulty to appreciate the areas with residual tumor by macroscopy. It is therefore mandatory to investigate a sufficient portion if not the whole tumor "bed" (the previous site of the tumor). In the following a proposal for standardized work up is provided.

Proposal for standardized work-up and reporting of TRG (modified from[6])

Photographic documentation

Photocopy or photograph of resection specimen (orientation and documentation of blocks and of histologically proven residual tumor)

Macroscopic description; tumor size (three-dimensional), distance to resection margins

Work-up

Inking of the deep (circumferential) resection margin

Complete embedding of the macroscopically identifiable tumor bed, orientated from proximal to distal in 0.5-cm levels. If tumor bed >8cm, significant regression in unlikely: first take blocks following the longitudinal and vertical largest dimension. If no or less residual tumor embed remaining tumorbed in second step. CRM is included in these blocks.

All slides stained by Hematoxylin/eosin, selected blocks by Periodic acid-Schiff, Elastica van Gieson staining; Immunohistochemistry may be helpful for discrimination of histiocytes and alterated tumor cells.

If no residual tumor: another three step sections to confirm complete response

Resection margins oral, aboral

Additional macroscopic findings

Lymph node stations. Immunohistochemistry (pan-Cytokeratin) if ypN0

Pathological report should include

UICC ypTNM status (including L, V, Pn)

UICC R-status

Grading, typing (according to WHO and Lauren for upper GI adenocarinomas)

Histopathological tumor regression grade (e.g. Becker Grade 1a, 1b, 2, 3).

Conclusion

Assessing tumor response to neoadjuvant treatment has been shown to be feasible by histopathological examination of the resected specimens in gastrointestinal carcinomas. It is highly recommended that TRG should be implemented in every histopathological report of neoadjuvant treated gastrointestinal carcinomas. However, there is a need for a simple, reproducible regression grading system with clear criteria. Both the pathologists´ and clinicians´ community have to work on standardization of specimen processing and reporting of TRGs.

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L 34

ROLE OF RAS AND BRAF ANALYSIS AND MMR IMMUNOHISTOCHEMISTRY IN TREATMENT DECISIONS IN COLORECTAL CANCER.

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Introduction

Recent guidelines for the management of colorectal cancer (CRC) recommend testing the mismatch repair (MMR) status in patients with stage II CRC if adjuvant treatment is considered[1] and testing RAS, BRAF mutation and MMR status in patients with metastatic CRC.[2]

The MMR proteins are involved in the correction of errors introduced in the microsatellites during replication of DNA. The inactivation of the MMR gene (MLH1, MSH2, MSH6, PMS2) by mutation or transcriptional silencing results in a deficient functioning of the MMR system and microsatellite instability (MSI). MSI is one of the two major types of genomic instability found in colorectal cancer carcinogenesis

RAS and BRAF are oncogenes involved in the EGFR signalling pathway. Mutation of RAS and BRAF are leading to a constitutive activation of the downstream MAPK pathway stimulating tumor cell proliferation, invasion, migration, and inhibition of apoptosis.

Treatment decisions include the answer to the question of whether treatment is needed and if so which treatment most likely will be successful. A molecular marker has utility in clinical practice if the marker is either prognostic, predictive or both. A prognostic biomarker provides information on the likely course of the cancer disease in an untreated individual. A predictive marker can identify subpopulations of patients who are most likely to respond to a given therapy. A prognostic marker helps the select the patient who needs treatment and a predictive marker helps to choose the most effective treatment for the patient.

I discuss here the utility of the MMR , RAS en BRAF analysis in the treatment decisions in stage II, III and IV colorectal cancer patients.

Stage II

Stage II disease includes tumors that have grown into the serosa (T3), visceral peritoneum (T4a), or next to or into nearby organs (T4b) but have no spread to nearby lymph nodes.

Stage II colorectal cancer has an good prognosis after complete surgery. However prognosis and risk of recurrence for patients with stage II CRC are inconsistent and notably the 5 year survival for patients with stage IIc is poorer than those with stage IIIa.[3]

It remains controversial if adjuvant treatment is beneficial.

Direct evidence from randomized controlled trials does not support the routine use of adjuvant chemotherapy for stage II patients.

The clinical challenge is to select patients who need treatment and to select the most appropriate treatment.

Although the evidence is limited guidelines recommend adjuvant treatment in high risk patients defined on clinicopathological characteristics: bowel obstruction, tumor perforation, T4 stage, poorly differentiated tumor, vascular, lymphatic or perineural invasion or less than 12 lymph nodes sampled.[1]

Risk assessment may be informed by evidence for MMR status. The most extensive analysis of the predictive and prognostic value of MMR status in stage II CRC was performed in the ACCENT database presented at ASCO 2014 by Dan Sargent.[4] The ACCENT Database is an collection of individual patient data of 26 randomized adjuvant colon trials presently more than 378000 patients. MMR data are available on 7803 patients from 17 trials. This gives the opportunity to examine relationship between MMR status and clinical endpoints based

on detailed individual patient data with clinical trial quality follow up. Compared to MMR proficient (MMRp) stage II CRC patients MMR deficient (MMRd) patients have a significant improved overall survival an time to recurrence in patients treated with surgery alone. Only patients with MMRp CRC and not those with MMRd tumors benefit from adjuvant fluoropyrimidine therapy and there is no significant association between MMR and prognosis in treated patients.

MMR is a prognostic marker in Stage II CRC but also a predictive marker while the treatment benefit of adjuvant 5FU is limited to stage II MMRp patients. When stage II patients are considered for adjuvant treatment MMR should be tested MMRd patients should not be recommended for treatment with fluoripyrimidines due to their excellent prognosis an lack of benefit of fluoropyrimidine treatment.

Stage III CRC

Stage III CRC includes tumors with spread to regional lymph nodes. Based on the degree of invasion of the intestinal wall and number of involved lymphnodes stage III is subdivided in Stage III A, B and C, the advanced stage having a worse prognosis.[3]

In patients with CCR stage III adjuvant treatment with fluoropyrimidine in combination with oxaliplatin is the standard of care. For patients who are older or intolerant to combination chemotherapy monotherapy with fluoropyrimidine either intravenous or peroral is the treatment of choice.[1] The efficacy of fluoropyrimidine monotherapy was confirmed in a meta-analysis based on pooled individual patient data from seven randomised trials. Adjuvant treatment results in a absolute benefit of 12% five year disease free survival and 7% overall survival.[5] The MOSAIC trial is the landmark study that led to the association of fluoropyrimidines with oxaliplatin as the standard of care. In this trial adjuvant chemotherapy with fluoropyrimidines was compared with the combination fluoropyrimidine and oxaliplatin. Although the absolute benefit in five year disease free survival is 7.5% the 6 year overall survival benefit remains modest (4.2 %).[6]

Because stage III CRC is an indication for adjuvant treatment with fluoropyrimidine in combination with oxaliplatin the clinical utility of analysis of MMR, RAS en BRAF status is limited. MMR is a prognostic factor in stage III colorectal cancer patients. Analysis of the ACCENT database shows an association of MMRd to improved survival in patients treated with surgery alone (not significant) and in surgery and 5FU treated patients (significant). Stage III CRC patients who have MMRd tumors do, in contrast with Stage II CRC patients, benefit from 5 FU adjuvant treatment.4 Prognostic value of KRAS an BRAF mutations in patients treated with FOLFOX was further analysed in the adjuvant stage III PETACC 8 trial.[7] This trial showed that the addition of cetuximab to FOLFOX[4] (leucovorin, fluorouracil, and oxaliplatin) did not improve disease-free survival in patients with KRAS exon 2 wild-type disease. No significant prognostic effect for disease free survival or overall survival was found for MMRd or BRAF mutation. KRAS mutation was significantly associated with shorter disease free survival and overall survival. Among patients with MMRp tumors, both KRAS mutation and BRAF mutation were independently prognostic for poorer disease-free and overall survival. Among those with MMRd tumors, KRAS mutation was not prognostic for disease-free or overall survival whereas BRAF mutation was associated with significantly longer disease-free survival and non significantly longer overall survival. The prognostic value of KRAS and BRAF mutation in patients treated with adjuvant FOLFOX in Stage III CRC is clearly depending on the MSI status.[7]

MMR , BRAF V600E or RAS mutation status is prognostic but does not currently alter CRC stage III patient management. However future adjuvant trials will have to take into account these molecular markers.

Stage IV

Stage IV colorectal cancer includes patients with distant metastasis.[3]

Management of patients with metastatic colorectal cancer involves surgery or other local and ablative therapies but most patients receive, during the course of their disease, treatment with chemotherapy in combination with monoclonal antibodies targeting epidermal growth factor receptor, vascular endothelial growth factor receptor or vascular growth factor.[2] Treatment strategy is composed of different lines in different combinations underscoring the need for predictive markers. However the predictive and prognostic markers at our disposal at the moment are limited to RAS, BRAF mutation and MSI analysis.

RAS

RAS mutations (KRAS exon 2, 3 and 4 and NRAS exon2, 3 and 4) occur in about 50% of metastatic CRC tumors. RAS mutation is a predictive marker for non respons to EGFR targeting monoclonal antibodies. The epidermal growth factor receptor (EGFR) is a member of the ErbB family. These receptors are transmembrane glycoproteins that consist of an extra cellular binding domain, a transmembrane domain and an intracellular domain with tyrosine kinase activity for signal transduction to areas downstream, signaling proteins involved into tumor cell proliferation, invasion, migration, and inhibition of apoptosis. The activating phosphorylation signal initiated by the intracellular active site of EGFR triggers the KRAS-BRAF-MEK-ERK pathway Receptor activation occurs when one of its ligands, the epidermal growth factor (EGF), the transforming growth factor-α (TGF-α), or amphiregulin, binds to its extracellular domain. Cetuximab and panitumumab are monoclonal antibodies that block the ligand binding site of the EGFR, thus inhibiting intracellular signalling. Cetuximab is a chimeric human-mouse antibody, while panitumumab is a fully humanized monoclonal anibody. EGFR-targeting monoclonal antibodies have proven efficacy in patients with metastatic cancer in combination with chemotherapy in first line, second line and third line. In third line they are also efficient in monotherapy. Mutations in KRAS (exon 2, 3 and 4) and NRAS (exon2, 3 and 4) are leading to a constitutive activation of the downstream MAPK pathway and predict a lack of response to EGFR-targeting monoclonal antibodies. So RAS mutation is a negative predictive factor that helps to define patients who will not respond to treatment with EGFR-targeting monoclonal antibodies. The evidence is produced by retrospective analysis of prospective randomised trials. The Prime trial was the first trial which showed that extended RAS mutations mutation predicts a lack of response to EGFR-targeting monoclonal antibodies.[8] In this first line colorectal metastatic trial patients were randomised between FOLFOX and FOLFOX in combination with panitumumab. Efficacy was in the primary analysis compared according to the KRAS-status. The primary end point was progression free survival. Banked tumor specimens were analysed for other RAS mutations and BRAF mutation: KRAS exon 3 (at codon 61) and exon 4 (at codons 117 and 146); NRAS exon 2 (at codons 12 and 13), exon 3 (at codon 61), and exon 4 (at codons 117 and 146); and BRAF exon 15 (at codon 600). RAS status was finally obtained in 1060 of the 1183 randomised patients. 512 (48%) were identified as having tumors with non mutated RAS (no KRAS or RAS mutations in exons 2, 3 and 4). 548 were identified as having a tumor with a mutated RAS. 108 (17%) patients originally categorised as KRAS wild had another RAS mutation. Patients without RAS mutations had a significant longer progression-free with panitumumab–FOLFOX4 versus FOLFOX4 alone. Patients with RAS mutations had an inferior progression-free survival and overall survival with panitumumab–FOLFOX4 treatment.

These results were confirmed in other EGFR targeting monoclonal antibody trials.

RAS testing should be carried out on all patients at the time of diagnosis of metastatic CRC and is mandatory before considering EGFR-targeting monoclonal antibody therapy.

BRAF mutation

BRAF mutation most commonly a valine to glutamic acid substitution of the 600th amino acid (V600E) is a negative prognostic factor. BRAF mutations occur in 5-10 % of metastatic CRC tumors and are mutually exclusive with RAS mutations.

In the PRIME trial patients with BRAF mutated tumors had an overall survival of less than one year and EGFR-targeted monoclonal antibodies had only limited, if at all, efficacy.[8] In a retrospective study BRAF mutant stage IV patients had a median progressive free survival of 6.3 months on first line chemotherapy and fewer patients went to further lines which were inefficient with a progression free survival of 2.5 months and this corresponds to the first time of restaging. BRAF mutated tumors do hardly ever respond to standard chemotherapy.[9] Because of early clinical decline patients with BRAF mutated CRC must be considered early after first-line therapy for clinical trials. If not available aggressive treatment with combination chemotherapy must be offered in frontline therapy to patients with a good performance status. Current clinical studies asses combinatorial approaches of multiple protein inhibitors.

Tumour BRAF mutation status should be obtained for prognostic assessment and potential selection for clinical trials.

MMR

MMRd occurs in 4-8% of tumors in metastatic CRC patient. Patients with MMRd metastatic CRC have a poor prognosis with worse progression free survival and overall survival than MMRp patients . This may be partially driven by the fact they have more frequent BRAF mutation. [10]

Recent data shows that MMRd predicts clinical benefit of immune check point blockade. CRC with MMRd have a greater number of mutations which results in a higher number of neoantigens that are potential immunogenic. The programmed death 1 (PD-1) pathway is a negative feedback system that represses Th-1 cytotoxic immune responses. The tumor inhibits the effector T-cell function by producing PD-L (programmed death ligand) that bind PD-1 expressed on the T-cell. By suppressing the immune surveillance PDl-1 expression on the tumor cells permits the neoplastic growth. Immune check point inhibitors are monoclonal antibodies that bind to PD-1 receptor or to PD-L1 ligand and block the PD pathway. Immune check point inhibitors have demonstrated antitumor activity in multiple advanced cancers and also there are preliminary data on efficacy in the treatment of colorectal cancer. Le et al reported a disease control rate 92 % in 13 patients with MMRd metastatic CRC with some durable responses while no patients with MMRp tumors responded to the treatment.[11] Although preliminary the results are so promising that at this moment MMR testing is recommended to select this small number of patient to include them in clinical trials with immune check point inhibitors.

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L 36

CERVICAL CYTOLOGY WITH LBC: (AB)NORMAL AND SOME RARITIES.

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October 15-2016; 9:45 hour; Hippo room, Program for Cytotechnologists

Introduction of liquid based cytology (LBC) in cervical cytology has resulted in different methods of preparing cervical smears and screening methods. Due to LBC continue controlled working processes are accomplished. This in combination with a standardized adequate staining procedure results in a constant high quality of the made smears without technical problems like air-drying and smear artifacts. Another advantage of using LBC is the ease of exchanging slides between different laboratories using the same (commercial) LBC method and the use of automated screening.

Much has been written about the best manner to screen a LBC cervical slide. Important is that this is done cautiously, because due to the clean background there exists a tendency to screen too fast. A quick prescreen at 4x objective is recommended in order to get a first impression followed by screening at 10x the whole slide In spite of this thin layer technique there will be still some overlap off cells and cell groups requiring careful examination.

At the start of working with a (commercial available) LBC method users must get used to small different morphological features in comparison with conventional smears. Due to wet fixation cells show less degenerative changes. They are more round up and show some shrinkage. The chromatin structure is far more easy to observe but must not be overrated hereby. Besides the squamous cells, endocervical epithelial cells are lying in more round up compact groups showing nuclear hyperchromasia. Endometrial cells display less degenerative changes too. The nuclei are more prominent and the chromatin can be better interpreted. With LBC detection of the common specific infectious diseases is comparable. In our practice it is less important due to the development of alternative microbiological techniques. In our practice detection of Candida is most common followed by Trichomonas infection.

In order to recognize neoplastic cells and discriminate between low and high SIL nuclear enlargement, shape and hyperchromasia besides the N/C ratio are still the most important criteria.

Solitary atypical cells can be true eye catchers in LBC slides. This may also apply for small individual high SIL cells. They can be more prominent and better to recognize as in the conventional screening method. As mentioned before the background is more clear and necrotic debris and mucus are less present. These elements are more located at the rim of the microscopic slide due to the LBC technique.

In general cervical cytology using LBC provides sufficient remaining material to perform a cell block. There are many known different methods, like working with agar or a commercial method like the automated cell blocker as Celliënt (Hologic). The advantage of using an additional cell block provides for the possibility of additional histochemical and immunocytological staining combined with possible additional morphological information. For example, a minimal deviation (low grade) adenocarcinoma can now be diagnosed by additional staining with strong positive staining of tumor cells with CEA and loss of ER expression. Besides primary cervical malignancies immunophenotyping of metastasis is possible. Mostly they are derived from the ovary, colon, bladder, kidney or breast. The presentation will show the cell changes and some rare cases.

INVITED LECTURES

L 37

CLASSIFICATION AND REPORTING OF APPENDICEAL MUCINOUS NEOPLASMS AND CARCINOMAS.

Joseph Misdraji, MD

WHO classification of epithelial appendiceal tumors[1]

o *Premalignant lesions*

- Adenoma
  - Tubular
  - Villous
  - Tubulovillous
- Dysplasia (intraepithelial neoplasia), low grade
- Dysplasia (intraepithelial neoplasia), high grade
- Serrated lesions
  - Hyperplastic polyp
  - Sessile serrated adenoma/polyp
  - Traditional serrated adenoma

o *Carcinoma*

- Adenocarcinoma
  - Mucinous adenocarcinoma
  - Low-grade appendiceal mucinous neoplasm
  - Signet ring cell carcinoma
- Undifferentiated carcinoma

o *Neuroendocrine neoplasms*

- Neuroendocrine tumor (NET)
  - NET G1
  - NET G2
- Neuroendocrine carcinoma (NEC)
  - Large cell NEC
  - Small cell NEC
- Mixed adenoneuroendocrine carcinoma
- EC cell, serotonin producing NET
- Goblet cell carcinoid
- L cell, Glucagon-like peptide-producing and PP/PYY producing NETs
- Tubular carcinoid

Classification of mucinous epithelial tumors

Adenoma

An adenoma is defined as a tumor that has intact muscularis mucosae. In the appendix, tubular adenomas in the appendix are rare, and most examples are villous. A diagnosis of adenoma implies that the lesion is cured by appendectomy. If there is any doubt, a diagnosis of LAMN is more appropriate. Adenomas can be graded as low and high grade, similar to colonic adenomas.

Low Grade Appendiceal Mucinous Neoplasm (LAMN)

LAMNs are appendiceal tumors that are characterized by a distinctive pattern of extension into the appendix wall that is presume to represent "pushing invasion". These low grade tumors line the appendix and appear as variably villous to undulating/flat mucinous epithelium with tall mucin vacuoles and variable atypia that in areas resembles low grade dysplasia in other parts of the GI tract. LAMNs are associated with alteration or destruction of the appendiceal muscularis mucosae, submucosa and muscularis mucosa, in the form of elastosis, hyalinization, and ultimately obliteration of the appendiceal landmarks. The epithelium grows on this fibrotic, hyalinized wall rather than on the usual lamina propria and muscularis mucosae. Furthermore, the tumor dissects, herniates, or even perforates through the wall of the appendix, all without eliciting the usual desmoplastic stromal reaction or invading as angular destructive glands typical of GI adenocarcinomas elsewhere.

Appendiceal tumors that have pushing invasion but high grade cytologic features are less common than LAMNs. In the PSOGI consensus process, the term that was proposed by this group for these tumors is high grade appendiceal mucinous neoplasm (HAMN). This term has not been adopted by the WHO, but appears in the CAP cancer protocol.

The prognosis of LAMN is highly dependent on the presence or absence of neoplastic epithelium outside the appendix.[2,3] LAMNs that are confined to the appendix, without extra-appendiceal mucin, are almost always cured by appendectomy. LAMNs associated with acellular mucin in the right lower quadrant carry a very low risk of recurrence of progression to pseudomyxoma peritonei whereas those that have mucinous epithelial cells in the mucin on the appendix serosa are at high risk of recurring.[4]

Adenocarcinoma

In the current nomenclature of appendiceal tumors, a diagnosis of adenocarcinoma requires infiltrative type invasion. Tumors with pushing invasion, even if they have disseminated to the peritoneal cavity or ovaries, are LAMNs. Adenocarcinomas are classified as mucinous adenocarcinoma, signet ring cell carcinoma, and adenocarcinoma not otherwise specified. In the appendix, adenocarcinomas are often of the mucinous variety, but intestinal type cancers and adenocarcinomas resembling pancreatic adenocarcinoma occur occasionally. Signet ring cell carcinomas also occur, and a subset of these might arise from a goblet ell carcinoid.

Reporting and staging of mucinous neoplasms

The staging for invasive adenocarcinoma of the appendix follows a protocol similar to colon cancer and is generally not difficult to perform. However, the staging for LAMN has been more confusing due to the unusual manner in which these tumors extend through the wall of the appendix. Several points deserve mentioning in the staging of LAMN.

1. Involvement of the appendiceal margin. When a LAMN is confined to the appendix, the status of the margin may dictate whether a surgeon decides to perform a cecectomy or right hemicolectomy. However, there is scant data on whether this is necessary. We examined a small series of cases and found that when additional surgery was performed, residual tumor was not found in the resection, and when additional surgery was not performed, the patients had benign follow up.[5] Therefore, based on this small series of cases, it appears that there is no compelling reason to perform additional surgery with a positive margin. However, the number of cases was small, and involvement of the margin was never florid; therefore, in cases of florid margin involvement, additional surgery might be prudent.
2. In the current AJCC staging system,[6] involvement of the appendix serosa and right lower quadrant qualifies as pT4a. Involvement of peritoneum beyond the RLQ qualifies as pM1a, and beyond the peritoneum qualifies as pM1b. Because the prognosis of a LAMN is entirely dependent on perforation and the presence of mucin and/or epithelial cells on the surface of the appendix, that must be clearly documented in the pathology report. Also, the grade of any mucinous tumor in the peritoneum must be clearly documented.
3. The grade of the tumor (and in particular the grade of the peritoneal tumor) is prognostically important. In fact, it is required to distinguish anatomic stage IVA from IVB.

Changes in the new AJCC TNM staging system, due out in 2017:

1. A T category was created specifically for LAMN. Tis (LAMN) is assigned to LAMNs that extend into muscularis mucosae. LAMNs that extend into subserosa or serosa are assigned a stage of T3 of T4, respectively. Tis (LAMN) was added to stage 0.
2. Involvement of the appendix serosa is still pT4a, but the "right lower quadrant" is deleted from the definition. Also, because it is recognized that abundant acellular mucin in the peritoneal cavity might be an ominous sign, acellular mucin within the peritoneum is assigned pM1a. Cellular mucin in the peritoneum is now pM1b, and beyond the peritoneal cavity is pM1c. Acellular mucin was added to the anatomic stage system.
3. A two-tier system for grading mucinous neoplasm was replaced by a 3-tier system modeled after Davison.

Mixed glandular and endocrine neoplasms

Nomenclature

Tumors with both glandular and endocrine differentiation are known as amphicrine tumors. In the appendix, these tumors are designated as goblet cell carcinoid. Related tumors with aggressive histologic patterns have been designated as either mixed carcinoid-adenocarcinoma or as adenocarcinoma ex goblet cell carcinoid.[7,8]

Goblet cell carcinoid

Goblet cell carcinoids are a distinctive type of tumor composed of clusters of goblet-like mucinous cells, endocrine cells, and a variable number of Paneth cells. These tumors usually appear as an area of circumferential thickening of the appendix, and thus may be recognized only upon microscopic examination of the specimen. Microscopically, goblet cell carcinoids show circumferential infiltration of the appendix by discrete nests of mucinous cells that resemble goblet cells or, in cases where the mucin compresses the nucleus to the edge of the cell, signet ring cells. Admixed with goblet-like or signet-ring like cells are cells with eosinophilic cytoplasm and occasionally Paneth cells.[8] The tumor typically does not elicit a stromal reaction.

Adenocarcinoma ex goblet cell carcinoid

In 1990, Burke et al defined carcinomatous growth patterns in goblet cell carcinoids as fused or cribriform glands, single file structures, diffusely infiltrating signet ring cells, or sheets of tumor cells.8 Tumors with < 25% carcinomatous growth were designated goblet cell carcinoid. Tumors with greater than 50% carcinomatous growth pattern were classified as mixed carcinoid-adenocarcinoma.

In 2008, Tang et al.[7] proposed a classification of goblet cell carcinoid tumors that distinguishes pure goblet cell carcinoid tumor from carcinomas that arise in association with them (carcinoma ex goblet cell carcinoid, signet ring type or poorly differentiated type). Adenocarcinoma ex goblet cell carcinoid, signet ring cell type demonstrates partial, or near complete, loss of goblet cell clustering as a major criterion. Adenocarcinoma ex goblet cell carcinoid, poorly differentiated carcinoma subtype, is defined as a goblet cell carcinoid tumor with at least one focus (occupying at least 1 low power field or 1 mm2) that is indistinguishable from a conventional poorly differentiated, or undifferentiated, adenocarcinoma. These carcinomatous areas resemble conventional gland forming adenocarcinomas of the GI tract or consist of sheets of malignant cells either with, or without, signet ring cell features, or show morphologic features of a high grade neuroendocrine carcinoma or undifferentiated carcinoma.

Two recent papers have validated the grading systems of both Burke and Tang. Taggart et al.[9] classified 74 goblet cell tumors and 68 adenocarcinomas without a goblet cell component, and found that the proportion of adenocarcinoma correlated with prognosis, but the histologic type of adenocarcinoma (signet ring or poorly differentiated) did not. Furthermore, they found that tumors with > 50% adenocarcinoma behaved similarly to poorly differentiated carcinomas without a goblet cell component. Thus, this study validates Burke's method of classifying adenocarcinomas arising in GCC. In contrast, Lee et al.[10] found that the Tang classification correlated with survival, but was difficult to apply. Instead, they proposed a two-tier grading system that they felt was easier to apply and correlated with prognosis. In their system, tumors are assessed 1 point for 1) cytologic atypia; 2) peri-tumoral stromal desmoplasia, and 3) solid growth pattern. Tumors with none of these or one of these features were classified as low grade, whereas tumors that had at least two features were classified as high grade.

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INVITED LECTURES

L 38

IGG4-RELATED DISEASE.

Mina KOMUTA (St.LUC-UCL university hospital, Brussels)

IgG4-related disease (IgG4-RD) is a fibroinflammatory condition that can affect variable organ systems. The key histopathological features are a dense lymphoplasmacytic infiltrate, storiform fibrosis, and obliterative phlebitis.

Diagnosis is based on a combination of clinical, biochemical, radiological and histological findings. However, clinically and radiologically, IgG4-RD often mimics malignant, infectious, and inflammatory diseases. In addition, serum IgG4 level is not always reliable. Therefore, the role of histopathology is crucial to diagnose IgG4-RD.

In this session, a general overview of IgG4-RD will be shown. Then after, IgG4-RD involving the liver, biliary tree, and pancreas will be discussed: IgG4-RD presents with diverse morphological changes, such as sclerosing cholangitis, inflammatory psuedotumor in the pancreas and/or liver, chronic active hepatitis, and autoimmune pancreatitis.

L 39

SURGICAL PATHOLOGY: SELECTED TOPICS.

TUMOR BUDDING IN THE DIGESTIVE TRACT.

Inti Zlobec

Tumor budding has gained considerable attention over the last decade as a clinically relevant feature in colorectal cancer and other tumors of the digestive tract. Defined as detached single cells or small tumor cell clusters at the invasion front, tumor buds are correlated with aggressive tumor behavior and unfavorable patient outcome. Although tumor budding is a recognized prognostic factor, its integration into daily diagnostic routine has been slow. Disagreements regarding the use of cytokeratin stains or H&E and the lack of consensus of scoring method have contributed to an on-going debate about the reporting of tumor budding. The reporting recommendations from the 1st International Tumor Budding Consensus Congress (ITBCC), which took place in April 2016 in Bern, Switzerland will help to provide a first guideline. Numerous immunohistochemical studies have underlined the similarities between the protein expression profiles of tumor buds and cells in an epithelial-mesenchymal transition (EMT), further underlining their aggressive nature. However, the microenvironment encapsulating these tumor buds is rich in other cells types like immune cells and cancer-associated fibroblasts and it is hypothesized that the balance of pro- and anti-tumor factors within this microenvironment tips the balance in favor of better or worse prognosis. Molecular studies on tumor buds and their environment are few, but will be necessary to further characterize the nature of these cells and their interactions. In this talk, the latest evidence on tumor budding as a clinically important feature will be outlined. Results from the ITBCC and the latest studies attempting to characterize tumor buds and their microenvironment will be presented. Together, this presentation should provide an overview on the latest developments affecting the field of tumor budding.

ORAL PRESENTATIONS

ORAL PRESENTATIONS

P 01

UCA1 OVEREXPRESSION IS A NEW INDEPENDENT PROGNOSTIC MARKER IN BLADDER CANCER ASSOCIATED WITH INCREASED PATIENT SURVIVAL.

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Introduction:

Non-coding RNAs have been shown to play important roles in carcinogenesis via complex mechanisms, including transcriptional and post-transcriptional regulation, as well as chromatin interactions. Urothelial-carcinoma-associated-1 (UCA1, a long non-coding RNA) was recently shown to have tumorigenic properties in urothelial bladder cancer (UBC), demonstrated by enhanced proliferation, migration, invasion and therapy resistance of UBC cell lines in vitro. These findings, mainly provided by in vitro cellular assays, suggest that UCA1 is associated with aggressive tumour behaviour and could have prognostic implications in UBC.

Methods:

Chromogenic in situ hybridization and immunohistochemistry were carried out on tissue-microarray to characterise UCA1mRNA, p53 and KI-67 expression in 208 UBC, including 145 non-muscle-invasive and 63 muscle-invasive cases. We investigated statistical relations between UCA1 expression and UBC pathological features, patient prognosis as well as expression of p53 and KI-67.

Results:

UCA1 was observed in the tumour cells of 166/208 (80%) UBC tested. No expression was noted in normal stromal and endothelium cells. Patients with UBC that overexpressed UCA1 had a significantly higher survival (p=0.006) compared to patients whose UBC didn't overexpress UCA1. This prognostic factor was independent of tumour morphology, grading, staging and concomitant CIS. In addition, the absence of UCA1 expression was associated with a high Ki67 proliferative index and two different patterns of p53 expression (strong nuclear expression or complete absence of staining).

Conclusions:

Our work identified UCA1 as a possible new independent prognostic marker in UBC that could play a pivotal role in bladder cancer carcinogenesis.

P 02

VASCULAR IMPACT ON DEMENTIA: THE ROLE OF CEREBRAL AMYLOID ANGIOPATHY AND MICROINFARCTION ON THE DEVELOPMENT OF DEMENTIA.

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Introduction:

Vascular dementia is a heterogeneous group of brain lesions and vessel disorders. Brain infarctions, microinfarctions, strategic infarctions and white matter lesions have been discussed to play a role. Several different vessels disorders and embolic events can cause these vascular brain lesions.

Aim:

The aim of this autopsy study is to evaluate the impact of vascular tissue lesions as well as of vessel diseases on cognitive decline in concert with Alzheimer (AD)-related pathology.

Methods:

We studied 212 human autopsy cases for the impact of vascular lesions and underlying vessel disorder in the development of dementing disorders. The cases were neuropathologically characterized for AD lesions, infarcts/microinfarcts, bleedings, the degree of atherosclerosis in the circle of Willis, cerebral small vessel disease, and cerebral amyloid angiopathy (CAA). Logistic and linear regression models were applied to clarify the interplay between the pathologies.

Results:

AD-related τ- and Aβ-pathology appeared to be the main variables to explain cognitive decline. Strategic infarcts/ microinfarcts in the CA1-subiculum region thereby contributed significantly in the development of dementia. Gross infarcts and lacunar infarcts had no major impact in the development of dementia. The only vessel disorder that on its own had effects on the dementia-related hippocampal microinfarcts was CAA of the capillary type (CAA-type 1) whereby CAA-affected capillaries were often found outside the infarct presumably exhibiting a potential prerequisite for hypoperfusion.

Conclusions:

Capillary CAA contributes to the development of dementia probably due to hypoperfusion-related hippocampal (strategic) microinfarcts. Thus, capillary CAA represents a vascular component of AD in the capillary CAA-related type of AD.

P 03

VASCULAR INSULIN-LIKE GROWTH FACTOR RECEPTOR TYPE 2 (IGF2R) EXPRESSION IS UPREGULATED IN MALIGNANT TUMOURS.

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Introduction:

Background: Insulin-like growth factor receptor type 2 (IGF2R) is a receptor belonging to the insulin-like growth factor (IGF) system. Involvement of IGF2R in the process of angiogenesis has been postulated in rare earlier studies. In previous work we demonstrated IGF2R expression in brain vessels and in particular in hyperplastic vessels of glioblastoma which is known as one of the most angiogenic malignancies. Since accumulating evidence suggests that tumoural angiogenesis is heterogeneous, we aimed to investigate whether this expression is restricted to glioblastoma vessels.

Aim:

We aimed to investigate the vascular expression of IGF2R in multiple types of tissues.

Methods:

Vascular IGF2R expression was evaluated by means of computer-assisted quantitative immunohistochemistry in tissue microarray sections from 17 colonic adenocarcinomas (ADC), 10 gastric ADC, 10 gastro-intestinal stromal tumours (GIST) , 20 pulmonary ADC , 15 pulmonary squamous cell cancers, 7 prostatic ADC, 4 renal tubulopapillary carcinomas, 14 renal clear cell carcinomas and 10 urothelial carcinomas. From these tumours, we also studied matched normal tissue except for GIST and urothelial carcinoma.

Results:

Summary of results: Our quantitative analysis revealed vascular expression of IGF2R in all tumours without difference between different tumour types. We also observed higher expression of IGF2R in tumoural vessels compared to normal vessels from the same patient (global p-value<0.001).

Conclusions:

This work reveals that IGF2R is expressed in blood vessels of different tumour types. Furthermore, our results suggest an upregulation of vascular IGF2R expression in malignant tumours.

P 04

USE OF IMMUNOHISTOCHEMISTRY AND NEXT GENERATION SEQUENCING FOR THE CLASSIFICATION OF GLIOBLASTOMAS.

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Introduction:

Glioblastomas (GBMs) are the most common primitive malignant tumors in adults. They are very heterogeneous tumors, whether it's about their morphology, prognosis or response to therapy. Last few years, studies using large scale analysis techniques established that GBMs present recurrent genetic alterations that allow their classification in several subtypes depending on their prognosis and response to therapy.

Aim:

We attempted to establish a molecular classification of GBMs that shall be easily applicable in clinical routine and also evaluated its impact on the patients' prognosis.

Methods:

We worked on a set of 59 GBMs samples, which were operated between 2013 and 2014, and received standard postoperative treatment (radiotherapy plus concomitant and adjuvant Temozolomide). On one hand we studied the expression of genes such as p53, EGFR, PDGFRa, vimentin and YKL40 by immunohistochemistry (IHC) (quantification and semi-quantification); on the other hand, we studied the mutational status of 50 genes and the copy number variations of 24 genes, sequenced by targeted next generation sequencing (NGS), using the Ampliseq Cancer Hotspot Panel on the Ion Torrent Personal Genome Machine.

We then compared the results obtained thanks to the two mentioned methods and evaluated the impact of the different studied factors on the patients' survival.

We also used the results to attempt to develop a classification of GBMs based on their protein expression profile (IHC) and their mutational and amplification profile (NGS).

Results:

We observed that PTEN mutation is associated with a decreased survival ($p = 0,043$).
We developed a classification with a prognostic value based on the presence of IDH1 mutation (determined by NGS) and the EGFR expression level (determined by IHC). Among IDH1 wild type patients, the group that highly expresses EGFR has longer survival.

Conclusions:

We established an algorithm which allows us to sort GBMs into 3 subgroups. These subgroups differ in prognosis and are based on their molecular profile obtained thanks to IHC and NGS, two techniques we can use in clinical routine.

P 05

IMMUNOHISTOCHEMICAL EXPRESSION STUDY OF ATRX, AN ALT SUPPRESSOR PROTEIN, IN INVASIVE BREAST CANCER, DUCTAL TYPE.

Leus H., Lefesvre P., Dhaene K., Forsyth R. / UZ Brussel, Brussels

Introduction:

Breast cancer, with the ductal type (BCa-DT) being most frequent, still is the leading cause of death in women. The immortal phenotype is a hallmark of cancer, including breast cancer. Cancer immortalization, either through telomerase re-activation or by the telomerase (TA) -independent Alternative Lengthening of Telomeres (ALT) process, is regarded a new target in cancer therapy. ALT activation, occurring in 15% of cancers, has been found to be robustly correlated with ATRX inactivating mutations and consequent loss of nuclear ATRX protein expression.

Aim:

In this study we looked for the presence or absence of ALT in BCa-DT using ATRX immunohistochemistry as surrogate marker.

Methods:

77 cases of invasive carcinoma of the breast, ductal type (74 ER/PR receptor positive and 3 triple negative cases) were retrieved from the archive, regardless of tumor diameter. FFPE material (50 punched-out surgical resection cases on 5 TMAs, 15 core biopsies, 11 whole mount surgical resections and 1 lymph node metastasis) was stained using an anti-ATRX monoclonal antibody (Sigma, clone CL0537, dil. 1/200) on the Ventana BenchMark XT automated immunostainer (OptiView detection system). Nuclei of normal endothelium and of the U-2 OS ALT cell line served as positive and negative controls respectively. Staining results were categorized in 'ATRX loss', 'ATRX indeterminate' and 'ATRX retained', corresponding to signal loss in >90% , 10-90% or <10% of tumor nuclei, respectively.

Results:

75/77 cases (97%) were classified 'ATRX retained', including all triple negative cases. Of these, 2/75 tumors showed nuclear signals of variable intensity, ranging from 1+ tot 3+. 2/77 cases (3%) were classified 'ATRX indeterminate' with max. 40% of nuclei entirely negative and variable signal intensity in positive nuclei. 0/77 (0%) case showed 0% signal in tumor nuclei ('ATRX loss').

Conclusions:

Unlimited replicative potential is a hallmark of cancer. Tumor cell immortalization occurs either through re-activation of TA or by activation of ALT. Both processes can be neutralized using TA- (Jäger, Genes 2016, 7, 39) or ALT-inhibitors (Flynn, Science 2015, 347 (6219):273). In order to predict BCa-DT response it is worthwhile dissecting immortalization processes.

ALT activation can be detected indirectly by (loss of) ATRX expression studies. Our study suggests that the majority of BCa-DT (97%) might be TA-driven. Since this percentage is higher than reported in the literature (75%), ALT-independency should be further confirmed by (positive) DAXX immunohistochemistry, another ALT suppressor gene product. No BCa-DT showed complete ATRX loss. In 4/77 (5%) cases discernible nuclear signal intensity variation was present. It needs further study to find out whether pathologic epigenetic changes can down-regulate ATRX expression quantitatively. Future studies will be conducted to see i) whether similar ATRX expression is present in the lobular type breast cancer, and ii) if neo-adjuvant chemotherapy influences expression rates.

P 06

CLINICAL APPLICATION OF TARGETED NEXT GENERATION SEQUENCING FOR COLORECTAL CANCER PATIENTS: A MULTICENTRIC BELGIAN EXPERIENCE.

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Introduction:

Colorectal Cancer is the second most frequent cancer in Europe irrespective of gender and is still yielding a high mortality rate. Despite broad screening program, 25% of the patients are metastatic at initial diagnosis. Promising targeted therapy and personalized medicine are making molecular profiling of tumours a priority. International efforts to catalogue mutations for multiple forms of cancer coupled with the successes of targeted agents in patients with molecularly defined tumors have generated enthusiasm for incorporating genomic profiling into clinical cancer practice. American and European guidelines are clearly emphasizing expanded RAS (KRAS and NRAS) status as a mandatory precondition for use of anti-EGFR therapy. Indeed, not only the benefit of anti-EGFR therapy is confined to RAS wild type (wt) tumours, but treatment with anti-EGFR antibodies may even harm patients with a RAS mutation. BRAF mutation is a strong negative prognostic biomarkers and evidence is accumulating that patients with a BRAF mutant tumour do not benefit from anti-EGFR therapy. Daily emerging new datas on theranostic and prognostic role of molecular biomarkers are a strong incentive for a validated, sensitive and broadly available molecular screening test in order to implement and improve multi-modal therapy strategy and clinical trials. Recently, next generation sequencing (NGS) has begun to supplant other technologies for genomic profiling that is now important for targeted therapies.

Aim:

In the present study, our goal is to share a successful 3 years-old clinical experience using NGS results to help therapeutical decisions.

Methods:

The Ion Torrent AmpliSeq colon/lung cancer panel which allows mutations detection in 22 cancer-related genes was prospectively used in clinical practice (BELAC ISO 15189 accredited method). The DNA of 750 colorectal tumours, including primary tumours and metastasis, from 11 different institutions was obtained from formalin fixed paraffin embedded material and subjected to targeted NGS using the Ion Torrent Personal Genome Machine.

Results:

Among the 750 tumours tested, 735 (98%) were successfully sequenced. The number of mutations per tumour ranged from 0 to 5 and 655 (89.1%) of the sequenced samples were harboring at least one mutation. 337 samples (45,8%) were KRAS mutant, with exon 2 mutation being the most common one (84%). 32 samples (4,3%) were positive for a NRAS mutation and 79 (10,7%) were BRAF mutant. The frequencies of these variants detected by NGS were consistent with frequencies reported in public databases. Moreover mutations and amplification in

potentially actionable genes were identified in 161 samples (21.9%) including 100 PIK3CA mutations (13.6%), 40 FBXW7 mutations (5.4%), 10 PTEN mutations (1.4%), 4 ERBB2 mutations (0.5%), 2 ERBB2 amplifications (0.3%) confirmed by FISH, 4 AKT1 mutations (0.5%) and 1 MAP2K1 mutation (0.1%). The median turnaround time between the reception of the sample in the laboratory and report release was 8 calendar days.

Conclusions:

Overall, the AmpliSeq colon/lung cancer panel can be applied in daily practice and provide reliable clinically relevant information for colorectal cancer patients.

POSTERS

POSTERS

POSTERS Morning Session:

POSTERS

POSTERS Afternoon Session:

P 07

IMMUNOHISTOCHEMICAL EXPRESSION STUDY OF ATRX, AN ALT SUPPRESSOR PROTEIN, IN SMALL CELL LUNG CANCER.

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/ UZ Brussel, Brussels

Introduction:

Small cell lung cancer (SCLC) is a dismal disease, with high proliferative capacity suggesting the presence of, hopefully drugable, immortalization mechanisms. 15% of cancers use telomerase (TA) -independent Alternative Lengthening of Telomeres (ALT) immortalization mechanisms. ALT activation has been found to be robustly correlated with ATRX inactivating mutations and consequent loss of nuclear ATRX protein expression.

Aim:

In this study we looked for the presence or absence of ALT in SCLC using ATRX immunohistochemistry as surrogate marker.

Methods:

FFPE material of 24 SCLC (21 primary tumors and 3 visceral metastases) cases was stained using an anti-ATRX monoclonal antibody (Sigma, clone CL0537, dil. 1/200) on the Ventana BenchMark XT automated immunostainer (OptiView detection system). Nuclei of normal endothelium and of the U-2 OS ALT cell line served as positive and negative controls respectively. Staining results were categorized in 'ATRX loss', 'ATRX indeterminate' and 'ATRX retained', corresponding to signal loss in >90% , 10-90% or <10% of tumor nuclei, respectively.

Results:

21/24 cases (88%), including 1 colon metastasis and 2 liver metastases, were classified 'ATRX retained'. Of these, 2 tumors showed nuclear signals of variable intensity, ranging from 1+ tot 3+. 2/24 cases (8%) were classified 'ATRX indeterminate' with 15% of nuclei entirely negative and variable signal intensity in positive nuclei. 1/24 (4%) case showed 0% signal in tumor nuclei ('ATRX loss'), with 3+ signal in intratumoral normal endothelial nuclei.

Conclusions:

Unlimited replicative potential is a hallmark of cancer. Tumor cell immortalization occurs either through re-activation of TA or by activation of ALT. Both processes can be neutralized using TA- (Jäger, Genes 2016, 7, 39) or ALT-inhibitors (Flynn, Science 2015, 347 (6219):273). In order to predict tumor response of SCLC it is worthwhile dissecting immortalization processes in (mostly) small SCLC biopsies. Activity of TA can only be measured in fresh protein lysates, requiring large tumor volumes. ALT activation on the other hand can be detected indirectly by (loss of) ATRX expression studies. Our study suggests that the majority of SCLC (88%) might be TA-driven, also being concordant with its epithelial nature. ALT-independency can further be confirmed by (positive) DAXX immunohistochemistry, another ALT suppressor gene product. Only 1 SCLC (4%) showed complete ATRX loss, and will be screened for inactivating mutations. This low frequency of loss of ATRX, a chromatin-remodeller, in SCLC, characterized by its condensed hyperchromatic nuclei, might be an argument against the hypothesis that closed heterochromatin predicts ALT activation. In 4/24 (16%) cases discernible nuclear signal intensity variation was present. It needs further study to find out whether pathologic epigenetic changes can down-regulate ATRX expression quantitatively, and whether the observed variation equals tumor ALT-TA mosaicism necessitating the administration of a cocktail of ALT- and TA-inhibitors.

P 08

IMMUNOMORPHOLOGY OF EWING'S AND OSTEOSARCOMA.

Vanhooren M. / UZ Brussel, Brussels

Introduction:

Osteosarcoma (OS) and Ewing's sarcoma (EWS) are the two most common sarcomas in childhood, principally arising as primary malignant tumors of bone and generally follow an aggressive clinical course, representing a major therapeutic challenge. Uniform neo-adjuvant chemotherapy has been applied since thirty years to all patients without further relevant progress since its introduction and is associated with significant acute as well as long-term morbidity in survivors. In general, novel outcome predictors at the time of diagnosis are highly warranted since commonly used parameters such as metastasis or response to neo-adjuvant chemotherapy reflect rather late disease stages and therefore do not allow early treatment changes. In contrast, tumor heterogeneity and non-standardized methods for marker detection and quantification are considered key drawbacks in the use of novel prognostic biomarkers in OS and EWS. Since analysis of the tumor microenvironment and especially the presence of an immune-infiltrate demonstrated its beneficial impact on survival in several other tumors combined with the hypothesis that analysis of the tumor microenvironment for OS and EWS outcome related biomarkers might be less dependent from the subtype, new steps should be undertaken in this field. In this study we made a first attempt to characterize this immunovascular microenvironment in both OS and EWS patients' specimens.

Aim:

Characterization of the immunovascular microenvironment in OS and EWS patients' specimens and correlation to clinico-pathological parameters

Methods:

FFPE material of 25 primary tumors (9 EWS, 16 OS specimens) was immunohistochemically stained using primary antibodies against immunovascular markers (CD31, D2-40, CD45, CD20, CD3, CD8) on the Ventana Benchmark XT automated immunostainer (OptiView detection system). The resulting H&E-stained whole sections of both cohorts where evaluated for the presence of different categories of tumor infiltrating lymphocytes (TILs), vasculature and lymphatics and scored semi-quantitatively. Subsequently, histologic findings were correlated with clinico-pathological variables after clinically characterizing both cohorts.

Results:

Disease-status at time of diagnosis, progression vs. no progression, age and tumor size were confirmed to be significant prognostic factors in both tumor types in univariate analysis.

None of the tissue sections in both cohorts showed any expression of D2-40. OS tissue samples showed to have a bigger expression of CD31, CD3 and CD8 compared to EWS tissue specimens. More CD8 staining was seen in patients presenting with local disease compared to patients presenting with metastatic disease at time of diagnosis as well as more CD8 staining in extraskeletal EWS lesions compared to intraskeletal EWS lesions. Neither of the immunovascular markers showed a statistically relevant impact on survival.

Conclusions:

Analyzing the immunovascular microenvironment, we discovered differences in vascularity and immune infiltrates considering different clinico-pathological variables, suggesting that this immunovascular microenvironment plays a role in disease development and evolution but no attribution of the overall immune infiltrate, nor vascularity, to outcome related parameters was statistically confirmed, suggesting no influence of the immunovascular microenvironment on outcome of these two different diseases and so questioning it's use in development of new therapeutic strategies or classification. Before drawing a definitive conclusion, one must take the little case number, especially for the EWS cohort to perform significant statistical analysis, in consideration, clearing the path for similar further investigations in bigger multi-centered studies.

P 09

PD-L1 EXPRESSION IN HUMAN ATHEROSCLEROTIC DISEASE: OF ANY IMPORTANCE?

Forsyth R. / UZ Brussel, Brussels

Introduction:

The significant contribution of T lymphocytes to atherosclerotic disease is well known. Moreover, the costimulatory and coinhibitory interactions between dendritic cells, macrophage antigen presenting cells and T-cells have been broadly investigated in animal models, however scarce data have been harvasted out of the human model.

Aim:

This study aims to investigate just a specific but important part of all these interactions, being the T cell inhibition in regulating its known pro-atherogenic T cell responses and this by the tissue expression of PD-L1 in plaques.

Methods:

Out of the archives of the pathology department of the Free University Hospital Brussels 30 biopsies concerning atherosclerotic disease were selected (15 end-arterectomy carotid and 15 stenotic aortic valve biopsies). As controls 5 non-pathological atherogenic aortic wall resections were included. Immunohistochemical staining with primary antibodies directed against CD3, CD8, PD-L1 and FOXP3 was performed using the Benchmark Ultra platform. All slides were digitalized and analyzed using the 3D-Histech and Pathomation platform. Statistical analyses were made by use of Excel and Wizard software.

Results:

CD3 and CD8 expressing T lymphocytes were easy to locate in these biopsies. These markers were more expressed in end erterectomy specimens when compared tot he aortic valves. Next tot his, , PD-L1 expression was mostly very limited and weak of signal. Of interest was that the fibroblasts of the neointima - covering the plaques - expressed PD-L1 more frequently and intense in these type of lesions. This expression was stronger in end-arterectomy specimens. FOXP3 was not expressed at all.

Conclusions:

PD-L1 expression has gained enormous interest in cancer models in view of immunotherapeutic interventions. PD-L1 expression in atherosclerotic disease has been a topic of interest, but mostly functionally limited to mice models. In this perspective, it is known that hypercholesterolemia enhances PD-L1 expression on splenic macrophages and dendritic cells of Ldlr-/- mice. Targeted deletion of PD-L1 lead to an increase in atherosclerosis and a higher presence of CD4 and CD8 expressing T-cells in these lesions. Above all, PD-1 deficient CD8 T-lymphocytes are capable to kill vascular cells, all illustrating the important role for coinhibition in surpressing effector CD8 T cells in the vascular wall. Taking our immunohistochemical PD-L1 expression into account together with extrapolating these known insights, highly indicates a protective mechanism of PD-L1 expression in the vessel wall of human. Moreover, the expression of PD-L1 by vascular fibroblasts is very suggestive as a surrogate partner for dendritic cells or antigen presenting macrophages in the coinhibitory interactions with atherosclerotic T-cells. Therefore particularly inhibiting further tissue damage in direction of the delicate intact overlying endothelial surface and, by this, sudden rupture of the plaque. Whether PD-L1 inhibition in cancer would enhance atheromatosis is still another major subject for further research.

P 10

OSTEOLYSIS IN NON-SARCOMATOUS METASTATIC BONE LESIONS: 'GRAFT VERSUS HOST'?

Forsyth R. , Wilgenhof K. / UZ Brussel, Brussels

Introduction:

Bone metastastic disease has not been extensively histologically. Bone metastases increase morbidity and mortality, where bone pain, pathological fractures and nerve compression are challenging to cure or even to control. Next to this, the general consensus is that polynuclear osteoclasts are the only cells that are able to digest the bone matrix, and therefore cause osteolytic lesions. However, osteoclasts are rarely found in routinely stained slides.

Aim:

The aim of this study is to investigate whether other cells, for example cancer cells, attribute to osteolysis. On the other hand, we wonder whether there may be a specific role for reactive – tumor associated - osteoclastic cells in the clinical setting of bone metastatic disease.

Methods:

29 cases of non-sarcomatous metastatic bone disease were selected. Anti-CD68 immunohistochemistry was performed to identify macrophages and osteoclasts, and to count the nuclei of osteoclasts. Anti-CD33, -CD16, and -CD51 immunohistochemistry was used to further characterize CD68-expressing cells.

Results:

Osteoclastic giant cells (containing 3 nuclei or more) were observed in 31% of the cases and large osteoclastic giant cells (10 nuclei or more) in 15% of the cases. A non-significant trend was found for osteoclasts containing five or more nuclei as a negative prognostic factor in the survival of these patients. It was demonstrated that bone metastases characterized by a peripheral distribution of CD33-expressing cells do predict a pathological fracture in 50% of cases.

Conclusions:

We confirmed that osteolysis is largely caused by osteoclastic cells. Moreover, CD68 and CD33 immunohistochemistry does contribute in determining the prognosis of patients with bone metastases. In other words, bone metastatic disease and its prognosis is the result of a close interplay between the graft (metastatic cells) and its host (tumor associated osteoclasts).

P 11

PD-L1 EXPRESSION IN GIANT CELL TUMOUR OF BONE: NEW TREATMENT OPTIONS BEYOND THE OSTEO-IMMUNOLOGICAL SYSTEM?

Forsyth R. , Leus H. / UZ Brussel, Brussels

Introduction:

Giant cell tumour of bone (GCTB) is a giant-cell rich neoplasm of which the interplay between neoplastic and reactive cells is key to its clinical behaviour. Since the introduction of denosumab a new era of medical treatment has arrived the scene of GCTB.

Aim:

As RANKL is also part of the osteo-immunology system, one could wonder whether another component PD-L1 does play a major role in the ethiogenesis and behaviour of GCTB.

Methods:

Thirty cases of GCTB were selected out of the archives of the Pathology Department of the Brussels Free University Hospital. Each case was revised by use of H&E slides. Next to this, immunohistochemistry was applied with antibodies directed against CD3, CD8 and PD-L1 (Ventana USA). Results were scored semiquantitatively (1+: max. 5%, 2+: between 6 and 25% and 3+: more than 25%) and analysed using the statistics program 'Wizard'.

Results:

CD3 and C8 immunohistochemistry showed a semiquantitative score of 1+ in all cases. Moreover, the T-lymphocyte density in these lesions was score as very low (2 to 3%) and in a few cases (n=6) no expression could be noticed. PD-L1 immunohistochemistry showed no expression on neoplastic spindle cells as on other non-lymphocytic cells.

Conclusions:

As GCTB is a neoplasm perfectly fitting into the osteo-immunological system, no true influence of T-lymphocytic infiltration could be noted. Moreover, using the immuno-checkpoint marker PD-L1 no expression could be detected in GCTB. Apart, from excluding this neoplasm from immunotherapy in a first glance, these findings are of most interest. As T-lymphocytes are known as an important source of RANKL-secretion, it becomes very clear that secretion of this ligand is more restricted to the neoplasm itself, illustrating the vicious circle of the osteoclastic and the neoplastic components of GCTB. Moreover, medical treatment seems not able to be expanded by anti-PD-L1 components and therefore has been restricted to denosumab untill this moment.

P 12

CASE REPORT

TWO PATIENTS UNDERGOING SURGERY FOR OESOPHAGOGASTRIC JUNCTION'S ADENOCARCINOMA WITH UNEXPECTED FINAL DIAGNOSIS.

Koopmansch C. , Demetter P. / UZ Brussel, Brussels

Content :

Our first case was a 67-year-old woman with abdominal pain who underwent oesophagogastric endoscopy incidentally revealing an erosion of the oesophagogastric junction. Our second case was a 83-year-old woman followed for gastritis, Barrett's oesophagus and Zenker's diverticulum who underwent oesophagogastric endoscopy showing the known Barrett's oesophagus but no other lesion. Based on biopsy, initial histological diagnosis of the two cases was in favour of an adenocarcinoma of the oesophagogastric junction. Case 1 underwent surgery and lower oesophagus resection was performed, while Case 2 underwent endoscopy and mucosectomy was performed. Based on the morphology and immunohistochemistry of the two surgical specimens, diagnosis of mixed adenoneuroendocrine carcinoma (MANEC) was finally made.

MANECs are defined as biphasic tumours exhibiting both exocrine (usually gastro-intestinal type adenocarcinoma) and endocrine components, with more than 30% of each component being represented. The NEC component displays the morphology and the immunophenotype described for pure NECs. Oesophageal MANECs are exceedingly rare; by consequence there are no recommended therapeutic strategies. Prognosis of MANECs largely depends on stage and proportion of each component, and in a retrospective series of NECs and MANECs, survival was better for patients with MANEC than for patient with pure NEC, most likely because of the higher stage observed in pure NECs compared with MANECs.

P 13

CASE REPORT

A UNUSUAL BILE DUCT TUMOUR IN A PATIENT WITH BILATERAL ADRENAL GLAND HYPERPLASIA.

Dehon R. (1), Driessens Natacha. (2), Demetter P. (2) /
[1] UCL Saint Luc, Brussels, [2] ULB Erasme, Brussels

Content :

A 70 years old Colombian woman, followed for a bilateral macronodular adrenal gland hyperplasia, presents with a biliary colic without cholestasis.

Clinically, the patient shows a Cushingoïd aspect and high blood pressure. Two of her four children have bilateral macronodular adrenal hyperplasia associated with Cushing's syndrome and one of her son has an ampullary tumour.

An abdominal CT scan shows a lesion located in the common bile duct with extension in the cystic duct. An endoscopic-ultrasound ponction of the bile duct suggests a gastrointestinal stromal tumour (GIST).

The tumor, initially stable, increases in size subsequently causing obstruction of the bile duct.

A Pet-CT shows a hypermetabolism of the distal common bile duct and the left adrenal gland. The values of CEA and CA19-9 are normal.

Surgical resection of the common bile duct and left adrenal gland is performed. The microscopic examination of the common bile duct indicates that the tumor is composed of "hepatoid cells", which are characterized by an eosinophilic cytoplasm, enlarged nucleus, and prominent nucleoli. The tumour cells are arranged in nests. The tumor infiltrates the soft tissue around the bile duct and a peri-biliary lymph node. The surgical margin is also infiltrated.

The immunostainings indicates that the tumour cells are positive for HepPar-1 and CDX2. CK7, CK19, PS100, chromogranin, synaptophysin are negative.

Based on the morphological findings and immunohistochemistry, this case is diagnosed as hepatoid carcinoma of the extrahepatic duct.

An additional genetic analysis has not revealed specific mutation.

After multidisciplinary discussion, adjuvant treatment with FOLFOX has been started.

In conclusion, this report presents a rare case of hepatoid carcinoma of the extrahepatic bile duct. This lesion is a rare type of adenocarcinoma that resembles hepatocellular carcinoma. Although the stomach is the most common location, other locations have been reported as in this case.

P 14

CASE REPORT

A YOUNG WOMAN WITH A THORACIC LOW-GRADE FIBROMYXOID SARCOMA.

Bienfait L. (1), Remmelink M. (1) , de Saint Aubain N. (2), Cappello M.(1) / [1] ULB Erasme, Brussels, [2] Institut Jules Bordet, Brussels

Content :

A 26-year-old woman showed a voluminous mass localised in the left posterior mediastinum. This mass was active on positron-emission tomography images, without any node extension. Radical excision was then performed. During the surgical act, a lung invasion, including pulmonary vein entrapment, was discovered and a lobectomy was necessary. Macroscopically, we observed a smooth and well-delimited mass, except near the lung. Microscopically, the tumour was composed of monomorph spindel cells entrapped in a vascularized stroma with area more cellularised and focal nodular. A highly MUC4 (a sensitive and specific immunohistochemical marker) expression was detected and the diagnosis of low-grade fibromyxoid sarcoma was finally performed (LGFMS). LGFMS is a rare soft tissue tumour with a relatively benign histology but apparently with a rather malignant attitude.

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