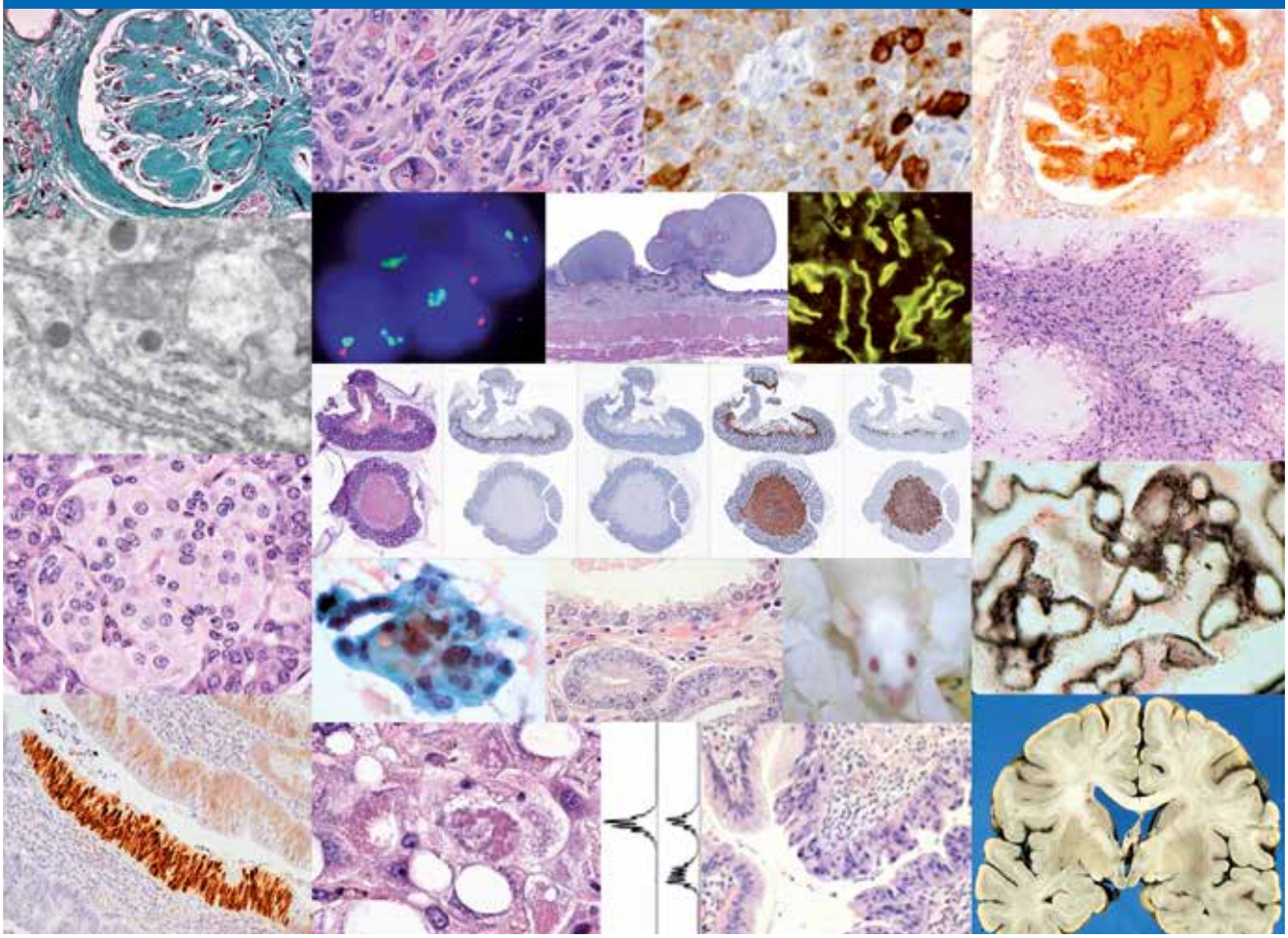
BWPath

BELGIAN WEEK OF PATHOLOGY

8th BELGIAN WEEK OF PATHOLOGY

OCTOBER 19-21, 2017

Congrescentrum Augustijnenklooster - Ghent



THURSDAY

FRIDAY

SATURDAY

www.belgian-society-pathology.eu

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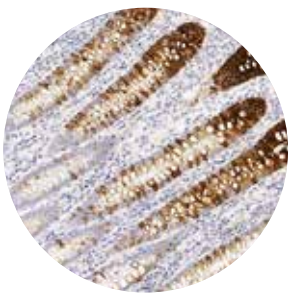

17218 Rev A · 08/2017

WELCOME

Dear Colleagues,

The previous seven editions of the Belgian Week of Pathology (BWP) were a huge success. Since several years this meeting takes 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, from October 19-21, 2017.

The Belgian Society of Pathology has undergone important changes, with the development of several working groups. All these groups have developed an up-to-date scientific program for the Eighth Belgian Week, accessible for experienced pathologists as well as for trainees and other young colleagues.

Internationally renowned experts and leading Belgian pathologists 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 breaks, lunches, the Thursday night drink and the Friday night cheese and wine reception.

This year's free paper and poster sessions take place on Friday. Best presentations will be awarded. Traditionally we welcome our cytotechnologists on Saturday morning.

The Ethics and Economy session will focus on costs and benefits of pathology, a difficult balance we all are confronted with. Especially in the framework of the changing landscape of Belgian medecine, the subject becomes more important than ever.

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 glad to see you at this Eighth Belgian Week of Pathology. Enjoy the meeting and the city!

Sincerely yours,

Pieter Demetter
BWP 2017 President

The Augustijnenklooster Library Visits

Visits are organised during all the Coffee Breaks of the Congress, you can register at the Desk in the morning or at lunchtime.

Departure of the visits: beginning of the Coffee Breaks from the Desk area, duration: 20min.

INDEX




GENERAL INFORMATION

Accreditation

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

Language

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

Abstracts

Authors were invited to submit abstracts until September 1, 2017.
The result of evaluation was sent to the first authors on september 15th

- Oral presentations will be presented during the Free paper Session on Friday from 16:00 to 17:30.
- Poster presentations will take place during the morning and afternoon coffee breaks of Friday October 20.

Posters will be displayed from Thursday 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 : Vogelenzangpark - 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
Marriott Hotel: Korenlei 10, 9000 Gent - Tel: +32 (0)9 233 93 93 / Fax: +32 (0)9 233 93 94

Event Coordinator

DME Events
Anne-France De Meyer – 57, Av. G. Demey – 1160 Brussels – Belgium
Tel : +32 477 27 00 45 / E-mail : anne.france.de.meyer@dme-events.eu

Ghent Tourism Office

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

BELGIAN SOCIETY OF PATHOLOGY

SBP-BVP Board :

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French-speaking Secretary :
Nicky D'Haene
Flemish-speaking Secretary :
Claire Bourgain
Treasurer :
Birgit Weynand

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Gert De Hertog
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Anne Hoorens
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Isabelle Salmon
Sofie Verbeke

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1. Working group of Cytopathology :

President : Birgit Weynand
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2. Working group of Digestive Pathology :

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4. Working group of Breast Pathology :

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5. Working group of Molecular Pathology :

President : Patrick Pauwels
Secretary : Nicky D'Haene

6. Working group of Gynecological Pathology

President : Jean Christophe Noel
Vice president : Claire Bourgain

7. Working Group of Urological Pathology

President : Thomas Gevaert
Vice president : Sandrine Rorive

BWP STEERING COMMITTEE

Belgian Week of Pathology 2017 : Steering Committee

President :

Demetter P.

Executive Secretary :

D'Haene N.

Councillors :

Bourgain C.

Hauben E.

Jouret-Mourin A.

Pauwels P.

Weynand B.

FOREIGN FACULTY

Adsay V.	Atlanta, USA
Bergeron C.	Cergy Pontoise, France
Berkhof H.	Amsterdam, The Netherlands
Croce S.	Bordeaux, France
Eycken K.	Lausanne, Swizerland
Flucke U.	Nijmegen, The Netherlands
Hasselblatt M.	Münster, Germany
Kristiansen G.	Bonn, Germany
Nayar R.	Chicago, USA
Rongioletti F.	Cagliari, Italy
Rubbia-Brandt L.	Geneva, Swizeland
Schmitt F.	Porto, Portugal
Serra-Arbeloa P.	Pamplona, Spain
Sheahan K	Dublin, Ireland
Schuuring E.	Groningen, The Netherlands
Slootweg P.	Nijmegen, The Netherlands
Stefanato C.	London, England
Thunnissen E.	Amsterdam, The Netherlands
Troncone G.	Napoly, Italy
Van de Vijver K.	Amsterdam, The Netherlands
Van Leenders G.	Rotterdam, The Netherlans

BELGIAN FACULTY

Bogers J.P.	Antwerp
Bourgain C.	Bonheiden
Creytens D	Ghent
Cuvelier C.	Ghent
D'Haene N.	Brussels
de Saint Aubain N.	Brussels
De Schepper S.	Ghent
Demetter P.	Brussels
Demols A.	Brussels
Dendooven A.	Antwerp
Eycken K.	Leuven
Floris G.	Leuven
Gevaert T	Leuven
Hauben E.	Leuven
Hérin M.	Gosselies
Jacomen G.	Duffel
Jouret-Mourin A.	Brussels
Lambein K.	Ghent
Lammens M.	Antwerp
Lelie B.	Knokke
Noël J.-C.	Brussels
Pauwels P.	Antwerp
Theunis A.	Brussels
Tombal B.	Brussels
Van den Bulcke M.	Brussels
Van den Eynden G.	Antwerp
Verbeke S.	Ghent
Weynand B.	Leuven

PROGRAM OVERVIEW

		KLOOSTERGANGEN ROOM Ground floor	AUGUSTIN ROOM Ground floor – 200 pax	HIPPO/CARTAGHUE Ground floor – 120 pax
THURSDAY October 19	9:00 - 10:00	Exhibition Area	Dermatopathology	Molecular Pathology
	10:00 - 10:30	Coffee Break Exhibition Area Poster	**Workshop SECTRA: Watch and Touch Digital Pathology**	
	10:30 - 12:00	Exhibition Area	Dermatopathology	Molecular Pathology
	12:00 – 12 :45	**LUNCH Exhibition Area / Posters**		**Satellite Symposium ASTRAZENECA 12:00 – 12:45**
	13:00 - 14:00			**Satellite Symposium MSD 13:15 – 14:00**
	14:00- 15:30	Exhibition Area	Digestive Pathology	Uropathology
	15:30 - 16:00	Coffee Break Exhibition Area Poster	**Workshop SECTRA: Watch and Touch Digital Pathology**	
	16:00 –17:15	Exhibition Area	Digestive Pathology	Uropathology
	17:15 - 18:15	**Welcome Drink** (See floorplan)		

PROGRAM OVERVIEW

THURSDAY

FRIDAY

SATURDAY

		KLOOSTERGANGEN ROOM Ground floor	AUGUSTIN ROOM Ground floor – 200 pax	HIPPO/CARTAGHUE Ground floor – 120 pax
FRIADAY October 20	9:00 - 10:30	Exhibition Area	Gynaecopathology	Tumours of Head & Neck
	10:30 - 11:00	Coffee Break Exhibition Area / Posters Session	**Workshop SECTRA: Watch and Touch Digital Pathology**	
	11:00 - 12:00	Exhibition Area	Gynaecopathology	Tumours of Head & Neck
	12:15 - 13:15	Exhibition Area	**Keynote Lecture :** R. Nayar / Chicago, USA	
	13:15 - 14:15	**LUNCH** Exhibition Area / Posters	**Workshop PHILIPS 13:30 – 14:15**	
	14:15 - 15:30	Exhibition Area	Breast Pathology	
	15:30 - 16:00	Coffee Break Exhibition Area / / Posters Session	**Workshop SECTRA: Watch and Touch Digital Pathology**	
	16:00 - 17:30	Exhibition Area	Breast Pathology	Free Paper Session
	17:30 – 17:45		**BWP 2017 Awards :** Boël Prize / Best Poster	
	17:45 –19:00	**Drink, Cheese & Wine** (See floorplan)		
SATURDAY October 21	9:00 - 10:00	Exhibition Area	Ethics & Economy: Costs & Benefits in Pathology	Program for Cytotechnologists
	10:30 - 11:15	Coffee Break Exhibition Area		
	11:15 - 12:45	Exhibition Area	Ethics & Economy: Costs & Benefits in Pathology	Program for Cytotechnologists
	12:45 - 13:00		Closing: P. Demetter	
	13:00 - 14:00	**LUNCH** Exhibition Area		General Assembly Belgian Society of Pathology 13:00 – 13:45

WATCH AND TOUCH
DIGITAL PATHOLOGY

INTERACTIVE TABLE FOR EDUCATION AND MDT- MEETINGS

The Table is an interactive learning and teaching tool that uses real anatomy and clinical cases to develop critical thinking in clinical training. Another useful application is gathering during the multidisciplinary team meetings around the table to discuss and better understand the radiology and pathology images and the hereon based diagnostic outcome.

INTERESTED HOW THIS INTERACTIVE TABLE WORKS?

Thursday 19th of October
1. 10:00 till 10:30 - Augustin Room
2. 15:30 till 16:00 - Augustin Room

Friday 20th of October
3. 10:00 till 10:30 - Augustin Room
4. 15:30 till 16:00 - Augustin Room

SECTRA

Knowledge and passion

THURSDAY 19 MORNING

Room Augustin

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

09:00-09:30: ***Vascular tumours.***
D. Creytens (Ghent)

09:30-10:00: ***New and old cutaneous mucinosis.***
F. Rongioletti (Cagliari, Italy)

10:00-10:30: ***Coffee break***
Workshop SECTRA: Watch and Touch Digital Pathology.

Visit of the Augustijnenklooster Library: 20 persons maximum. Please register at the Desk.

10:30-11:15: ***Hair pathology.***
C. Stefanato (London, U.K.)

11:15-11:45: ***Cutaneous metastases.***
N. de Saint Aubain (Brussels)

Room Hippo

09:00-12:00: **Molecular pathology**
Chairpersons: P. Pauwels (Antwerp), N. D'Haene (Brussels)

09:00-09:30: ***Molecular aspects of the latest WHO classification of lymphomas.***
A. Dendooven (Antwerp)

09:30-10:00: ***Can molecular biology improve the diagnosis of thyroid nodules?***
N. D'Haene (Brussels)

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

Visit of the Augustijnenklooster Library: 20 persons maximum. Please register at the Desk.

10:30-11:00: ***Molecular pathology and testing of brain tumours.***
M. Hasselblatt (Münster, Germany)

11:00-11:30: ***Lung cancer : beyond EGFR and ALK.***
E. Schuuring (Groningen, The Netherlands)

11:30-12:00: ***PD-L1 in lung cancer.***
E. Thunnissen (Groningen, The Netherlands)

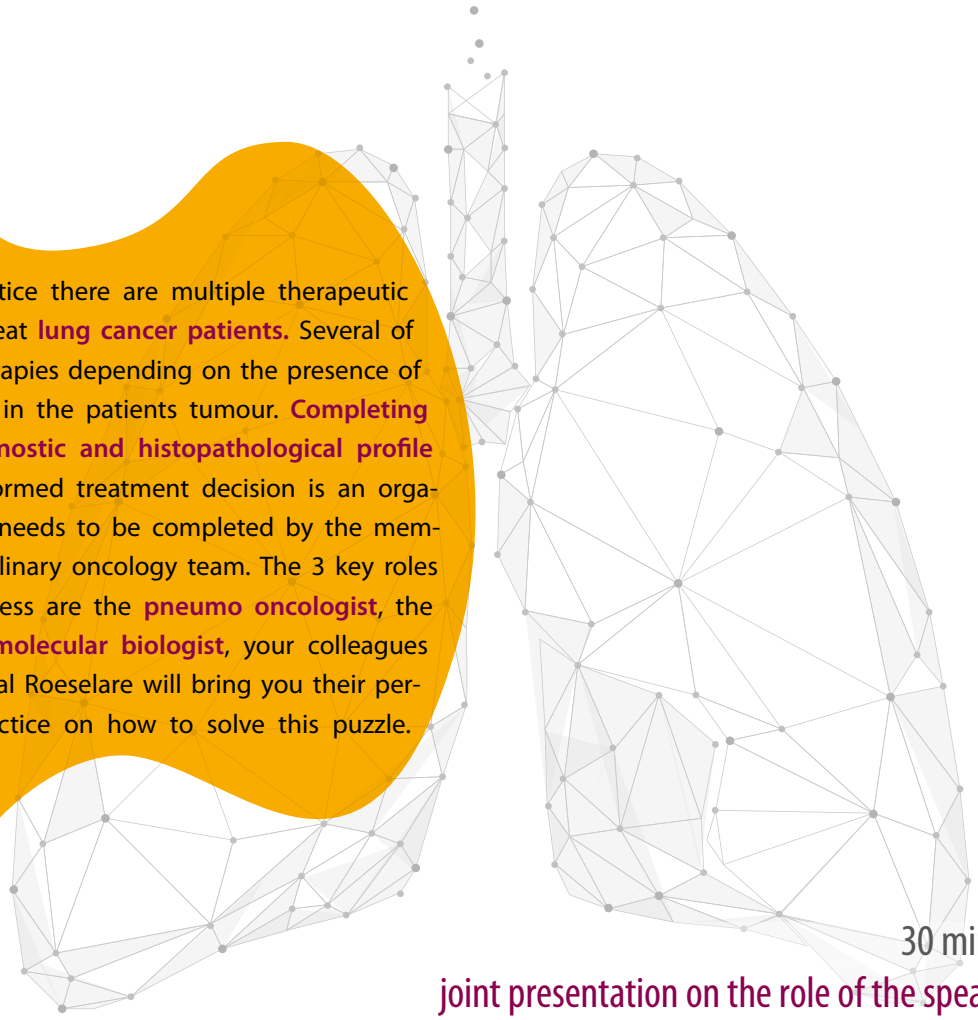
12:00-14:00: ***Lunch***

AstraZeneca

AstraZeneca satellite symposium
at the Belgian Week of Pathology

Thursday 19 October 12:00-12:45

Therapy choices for the NSCLC patient today: solving the testing **puzzle** in the multidisciplinary team at AZ Delta



In todays clinical practice there are multiple therapeutic options available to treat **lung cancer patients.** Several of them are targeted therapies depending on the presence of diagnostic biomarkers in the patients tumour. **Completing a full molecular diagnostic and histopathological profile** that allows a well informed treatment decision is an organisational puzzle that needs to be completed by the members of the multidisciplinary oncology team. The 3 key roles in the diagnostic process are the **pneumo oncologist**, the **pathologist** and the **molecular biologist**, your colleagues of the AZ Delta hospital Roeselare will bring you their perspective and best practice on how to solve this puzzle.

30 minutes:
joint presentation on the role of the speaker in the multidisciplinary team

15 minutes:
panel discussion with the audience

Location
Augustijnenklooster,
Academiestraat 1,
9000 Gent

Room Hippo/Cartague

Lunch will be available in the room

Speakers

Dr. Dedeurwaerdere Franceska Laboratorium voor pathologie;

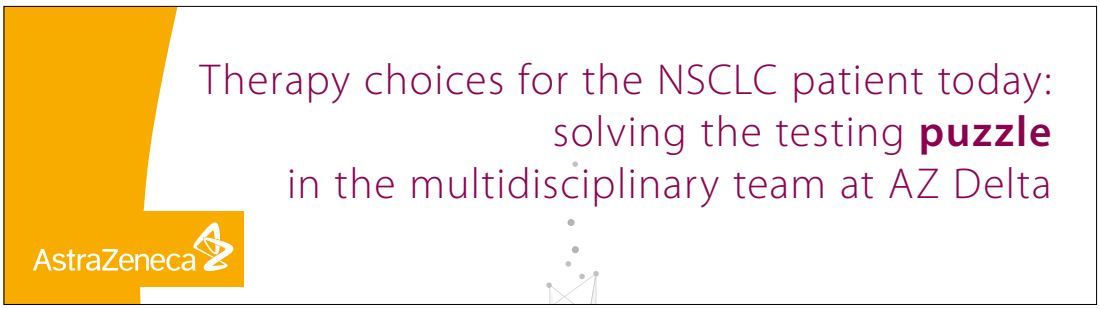
Prof. Dr. Martens Geert Dienst Laboratoriumgeneeskunde;

Dr. Demedts Ingel Thoracale Oncologie

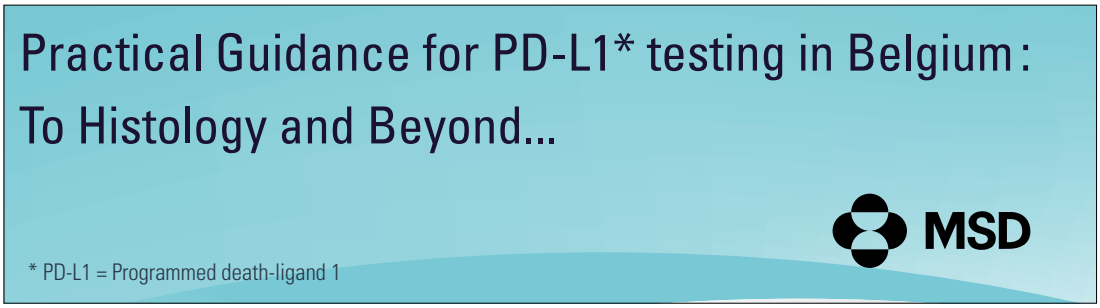
THURSDAY 19 AFTERNOON

Room Hippo

12:00-12:45: Satellite Symposium ASTRAZENECA



13:15-14:00: Satellite Symposium MSD



Room Augustin

14:00-17:15: **Digestive Pathology**
Chairpersons: A. Jouret-Mourin (Brussels), C. Cuvelier (Ghent)

14:00-14:45: ***Personalised treatment for patients with gastrointestinal stromal tumours : what clinicians expect from pathologists.***
A. Demols (Brussels)

14:45-15:30: ***Intrahepatic vascular diseases: revisiting and evolving.***
L. Rubbia-Brandt (Geneva, Switzerland)

15:30-16:00: ***Coffee break***
Workshop SECTRA: Watch and Touch Digital Pathology.

Visit of the Augustijnenklooster Library: 20 persons maximum. Please register at the Desk.

16:00-16:30: ***Drug-induced gastrointestinal pathology.***
K. Sheahan (Dublin, Ireland)

16:30-17:15: ***Precursor lesions of carcinomas of the gallbladder and bile ducts: increasingly recognised diseases.***
V. Adsay (Atlanta, U.S.A.)

8th Belgian Week of Pathology

19-21 October 2017, Ghent

Practical Guidance for PD-L1* testing in Belgium: To Histology and Beyond...

Thursday, 19 October 2017

13:15 – 14:00

Satellite Symposium
Sponsored by MSD

Augustijnenklooster Ghent
Hippo / Carthague

FACULTY

Nicky D'Haene MD, PhD
Professor, Dept. of Pathology. Hôpital Erasme, Université Libre de Bruxelles (ULB), Brussels, Belgium

Birgit Guldhammer Skov MD, DMSc
Assoc. Professor, Dept. of Pathology. University Hospital, Rigshospitalet, Copenhagen, Denmark

AGENDA

13:15 - 13:20	Introduction	Nicky D'Haene
13:20 - 13:35	Update of the Belgian Guidelines for Pathological and Molecular Diagnosis in Non-Small-Cell Lung Cancer	Nicky D'Haene
13:35 - 13:55	Paired Comparison of PD-L1 Expression on Cytologic and Histologic Specimens from Malignancies in the Lung	Birgit Guldhammer Skov
13:55 - 14:00	Q&A	

* PD-L1 = Programmed death-ligand 1

MSD Belgium SPRL/BVBA Clos du Lynx, 5, Lynx Binnenhof – Bruxelles 1200 Brussel

ONCO-1232236-0000 • Date of last revision: 09/2017

MSD ONCOLOGY

THURSDAY 19 AFTERNOON

Room Hippo

14:00-17:00: **Uropathology**
Chairpersons: T. Gevaert (Leuven), S. Verbeke (Ghent)

14:00-14:45: ***Biomarkers in prostate cancer.***
(G. Van Leenders (Rotterdam, The Netherlands)

14:45-15:30: ***Emerging entities in prostate cancer: intraductal carcinoma and neuro-endocrine differentiation.***
G. Kristiansen (Bonn, Germany)

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

Visit of the Augustijnenklooster Library: 20 persons maximum. Please register at the Desk.

16:00-16:30: ***Belgian checklists for prostate cancer (biopsy and RALP).***
T. Gevaert (Leuven)

16:30-17:00: ***Is there a role for the pathologists in guiding the treatment of metastatic prostate cancer?***
B. Tombal (Brussels)

Room Kloostergangen

17 :15-18 :15 : **Welcome Drink**

THURSDAY

Welcome Drink

THURSDAY 19 AFTERNOON

17 : 15 - 18 : 15

IN THE EXHIBITION AREA

FRIDAY 20 MORNING

Room Augustin

09:00-12:15: **Gynaecopathology**
Chairpersons: J.-C. Noël (Brussels), C. Bourgain (Bonheiden)

09:00-10:00: ***Uterine smooth muscle, endometrial stromal tumours and UTROSCT: state of the art and perspectives.***
S. Croce (Bordeaux, France)

10:00-10:45: ***Coffee break***
Workshop SECTRA: Watch and Touch Digital Pathology.

Visit of the Augustijnenklooster Library: 20 persons maximum. Please register at the Desk.

10:45-12:15: ***Slide seminar.***
J.C. Noël (Brussels), C. Bourgain (Bonheiden)

G. Jacomen (Duffel), M. Hérin (Gosselies), K. Van de Vijver (Amsterdam, The Netherlands)

12:15-13:15: **Keynote lecture**
Chairperson: B. Weynand (Leuven)

From Bethesda to Paris at LAST: the value of standardised terminology.
R. Nayar (Chicago, U.S.A.)

Room Hippo

09:00-12:15: **Tumours of head and neck: what's new in the 2017 WHO classification?**
Chairpersons: M. Lammens (Antwerp), E. Hauben (Leuven)

09:00-09:30: ***HPV-related tumours in the 2017 WHO classification and differential diagnosis.***
E. Hauben (Leuven)

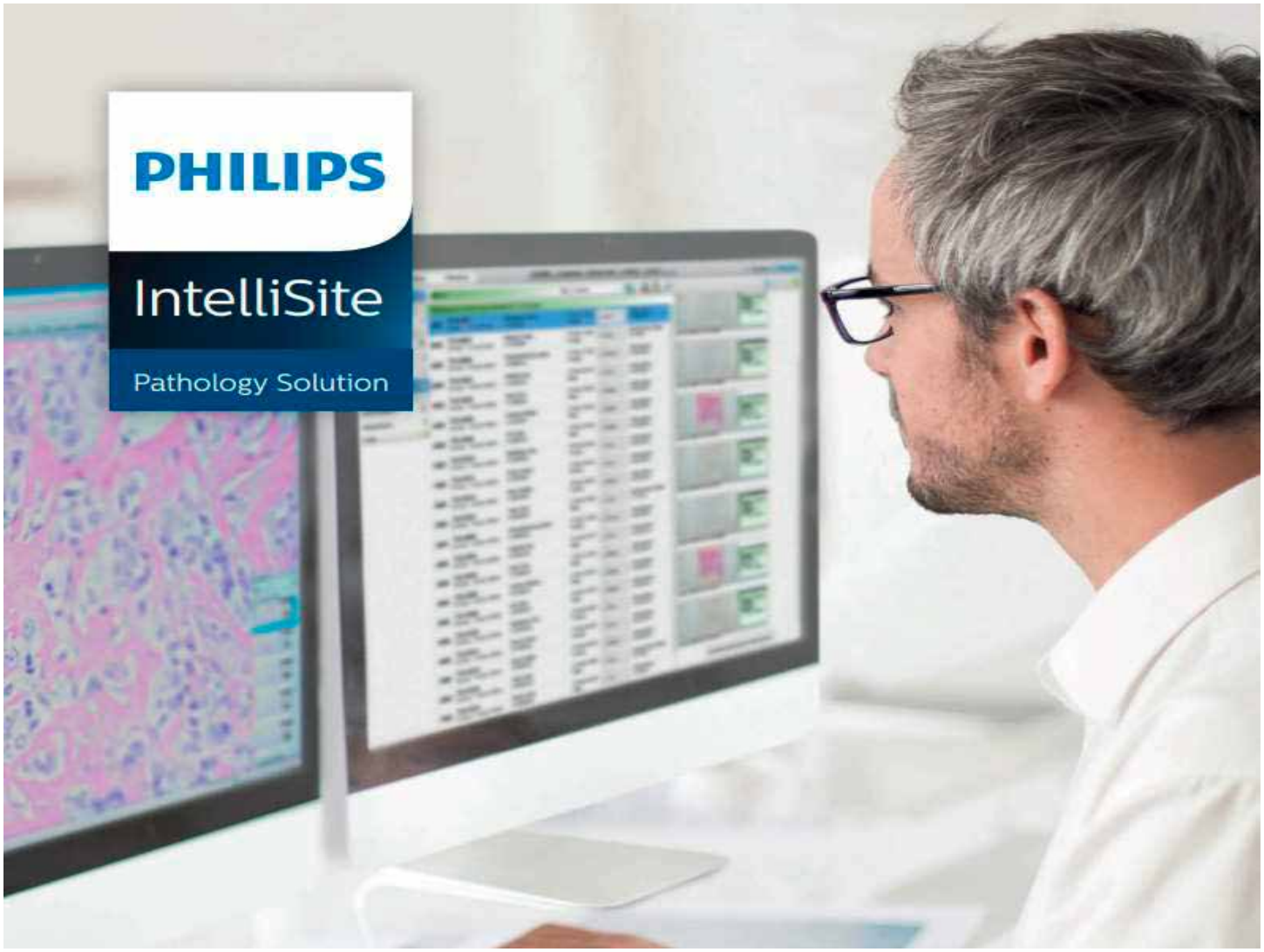
09:30-10:00: ***Sinonasal tumours.***
M. Lammens (Antwerp)

10:00-10:45: ***Coffee break***

Visit of the Augustijnenklooster Library: 20 persons maximum. Please register at the Desk.

10:45-11:30: ***Odontogenic and maxillofacial bone tumours.***
U. Flucke (Nijmegen, The Netherlands)

11:30-12:15: ***Tumours of the salivary gland.***
P. Slootweg (Nijmegen, The Netherlands)



Connecting you with cases and colleagues

Discover more…
Friday 20th of October
13.30 – 14.15 Augustin Room

The Image Management System (IMS) viewer is our case viewing & management software 'designed for pathologists with pathologists' to get you through cases as fast as possible. The IMS viewer is part of the Philips IntelliSite Pathology Solution. It provides easy access to information and resources, thereby enabling better-informed decisions.

The IMS viewer is your gateway to review and analyze digitized images. Comprehensive and flexible workflow tools for routine diagnosis give you an advantage over traditional methods of case organization.

Key advantages

- *Real-time collaboration and ergonomic design*
- *Case assessment tools for remote viewing and case presentation*
- *Case centric work list for high performance and balanced workload*
- *Flexible case organization on personal and department level*

FRIDAY 20 AFTERNOON

13:15-14:15: ***Lunch***

Room Augustin

13:30-14:15: ***Workshop PHILIPS: Overview of specific digital tools for the pathologist in a routine clinical workflow.***

14:15-17:15: **Breast pathology**
Chairpersons: G. Van den Eynden (Antwerp), K. Lambein (Ghent)

14:15-15:00: ***Histological subtypes of TNBC.***
K. Lambein (Ghent)

15:00-15:30: ***Salivary gland type tumours of the breast.***
G. Floris (Leuven)

15:30-16:00: ***Coffee break***
Workshop SECTRA: Watch and Touch Digital Pathology.

Visit of the Augustijnenklooster Library: 20 persons maximum. Please register at the Desk.

16:00-16:45: ***Tumour-infiltrating lymphocytes and PDL-1 in breast cancer.***
F. Schmitt (Porto, Portugal)

16:45-17:15: ***BRCA and BRCA-like genomic patterns in breast cancer: benefit of high-dose platinum-based chemotherapy.***
K. Van de Vijver (Amsterdam, The Netherlands)

Room Hippo

16:00-17:30: **Free Papers**
Chairpersons: P. Demetter (Brussels), C. Bourgain (Bonheiden)

16:00 **P 01** Immunohistochemical study of the ATRX/DAXX/H3.3-based alt pathway in pleural mesothelioma.
M. Vanhooren, P. Lefesvre, R. Forsyth, K. Dhaene / UZ Brussel

16:15 **P 02** Histopathological features of metastatic breast carcinomas carrying somatic mutations in ERBB2.
L. Jongen (1), H. Wildiers (2), D. Lambrechts (3), A. Laenen (4), P. Neven (2), G. Mann (6), R. Cutler Jr. (5), A. Lalani (6), G. Floris (6) / [1] KU Leuven, [2] KU Leuven - Oncology, [3] KU Leuven - Human Genetics, [4] Interuniversity Centre for Biostatistics and Statistical Bioinformatics, Leuven; [5] Puma Biotechnology, Los Angeles, USA, [6] KU Leuven - Imaging and Pathology

FRIDAY

16:30 **P 03** High PD-L1 expression in inflammatory breast cancer is associated with b-cell infiltration.
M. Parizel (1), C. Van Berckelaer (2), P. Van Dam (1), M. Kockx (3), S. Van Laere (2), M. Baldewijns (1), C. Colpaert (4) / [1] UZ Antwerpen, [2] TCRU CORE U Antwerpen, [3] Histogenex, [4] GZA Ziekenhuizen Campus Sint-Augustinus, Wilrijk

16:45 **P 04** Case Report: Case Report of an unusual nodule on the skin of the nose.
G. Broeckx (1), V. Siozopoulou (1), P. Pauwels (2) / [1] UZ Antwerpen, [2] Working group EQA, Commission of anatomic pathology, Brussels

17:00 **P 05** Contribution of proliferation and invasion criteria to the prediction of recurrence of non-functioning pituitary adenomas: retrospective analysis of 120 cases.
J. Lelotte(1), C. Raftopoulos(1), A. Jouret-Mourin(1), A. Michotte(2), D. Maiter(1)/ [1] UCL Saint-Luc, Bruxelles, [2] UZ Brussel

17:15 **P 06** Case Report: A young woman with a small cell carcinoma of the ovary, hypercalcemic type, large cell variant.
H. Leus (1), K. Deraedt (2), S. Sahebali (1), P. De Sutter (1), R. Forsyth (1) / [1] UZ Brussel, Brussel; [2] UZ Leuven

17:30-17:45: **BWP 2017 Awards**

Boël Prize: *Best Oral Presentation*

Belgian Society of Pathology Prize: *Best Poster*

Room Kloostergangen

17 :45-19 :00 : **Cheese & Wine**

We'll be pleased to welcome you at our

Cheese *wine & JAZZ*

FRIDAY

BWPath

BELGIAN WEEK OF PATHOLOGY

FRIDAY 20
AFTERNOON

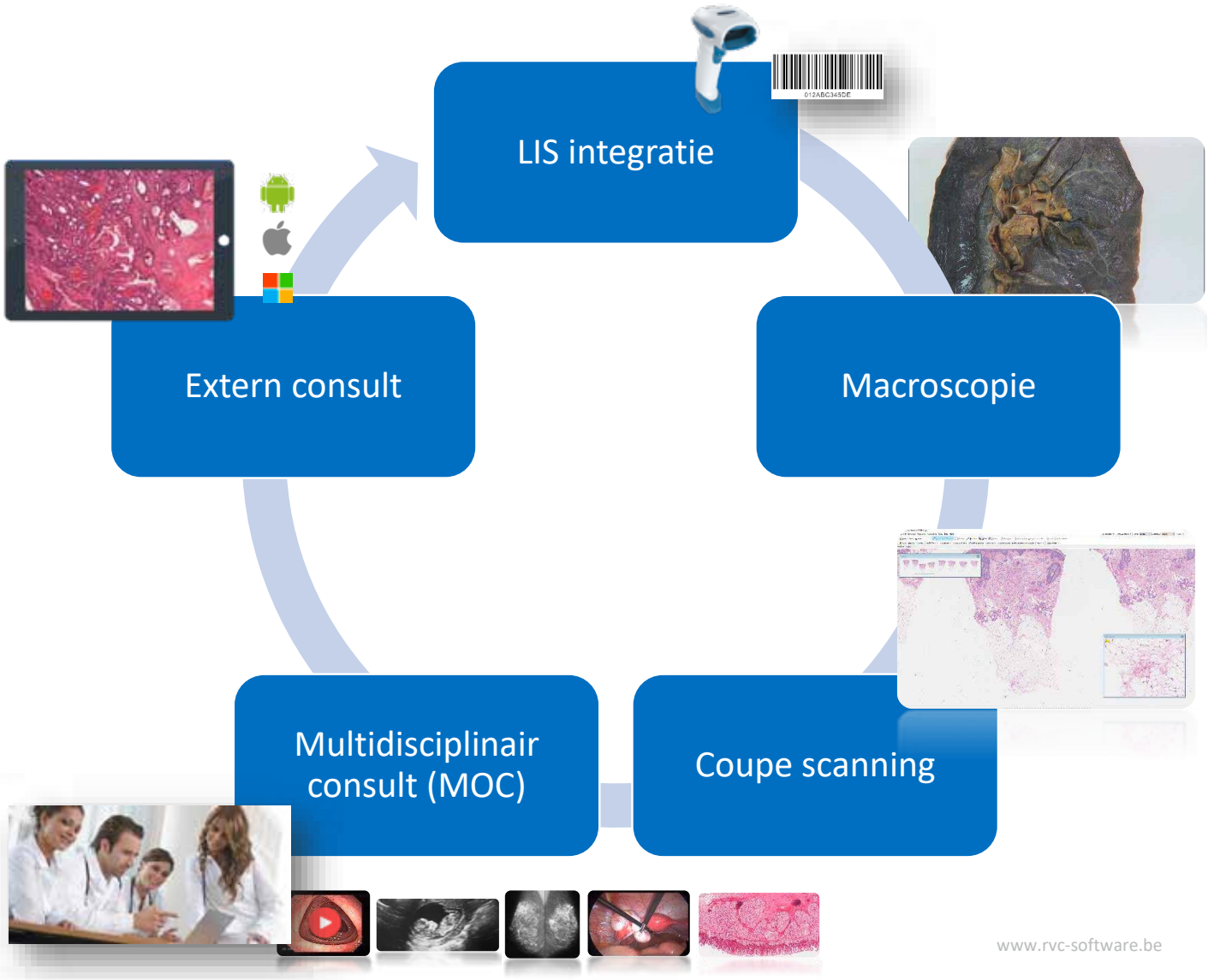
17 : 45 - 19 : 00

IN THE EXHIBITION AREA

software
for healthcare

RVC Clinical Assistant

Macroscopie
Beelden van alle merken scanners
Streaming en panels



Room Kloostergangen

Poster Session

Friday Ocotber 20: during the morning and afternoon coffee breaks.

P 07 Soluble amyloid β-protein is associated with increasing τ-pathology in app-tau transgenic mice.
D. Thal (1), S. Hipp (2), A. Rijal Upadhaya (2), K. Balakrishnan (2), J. Reichwald (3), S. Rabe (3), M. Faendrich (2), M. Staufenbiel (3) / [1] UZ Leuven, [2] University Hospital Ulm, Germany, [3] Novartis, Basel, Switzerland

P 08 Routine histopathological examination of mastectomy specimens after gender-confirming surgery.
S. Van Renterghem (1,2), J. Van Dorpe (2), S. Monstrey (2), J. Defreyne (2), K. Claes (2), M. Praet (2), S. Verbeke (2), G. T'Sjoen (2), M. Van Bockstal (2) / [1] CUSL, Brussels, [2] UZ Gent

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C. Koopmansch, R. Düttmann / CHU BRUGMANN, Brussels

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L. Verheuen (1), M. Baldewijns (2), F. Eyskens (2), C. Colpaert (2) / [1] AZ Sint Jan Brugge; [2] UZ Antwerp

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S. Bouri, V. Bogne, JC. Noël, L. Verset / ULB Erasme,Brussels

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L. Bienfait, A. Buggenhout, T. Quackels, L. Verset, P. Demetter / ULB Erasme, Bruxelles

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L. Vanwalleghem (1), E. Beerens (2), K. Dhaene (3), F. Dôme (4), D. Hoton (5), M. Praet (6), M. Remmelink (7), H. Van De Walle (8), J. Van Goethem (9), L. Vanwalleghem (1), E. Verbeken (10), B. Weynand (5) / [1] AZ Sint Jan Brugge, [2] AZ Nikolaas, Sint-Niklaas, [3] VUB, Elsene, [4] ULG, Liège, [5] CHU UCL Mont Godinne, [6] UZ Gent, [7] ULB Erasme, Brussels, [8] LABORATOIRE CMP, Brussels, [9] ZNA Middelheim, Antwerpen; [10] UZ Leuven

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L. Bienfait (1), B. Doukoure (2), N. D'Haene (1), I. Salmon (1), C. Decaestecker (1), P. Demetter (1), L. Verset (1) / [1] Hôpital Erasme, Bruxelles; [2] UFR Sciences Médicales d'Abidjan, Abidjan, Ivory Coast

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P 26 Case Report: Association of an ovarian adult granulosa cell tumour and endometrioid intraepithelial neoplasia: a case report and comprehensive review of literature.
Q. Fontanges, K. El Ali, J.C. Noël / ULB Erasme

P 27 A systematic review about the positive predictive value of high-grade squamous intraepithelial lesion on cytology for the histological diagnosis of cervical intraepithelial neoplasia 2 or more.
A. Van Loon (1), N. Karia (1), I. Benoy (1), C. Simoens (1), J. Bogers (2) / [1] AML bvba, Antwerp, [2] U Antwerp

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A. Camboni, I. Ferreira, M. Komuta / UCL Saint-Luc, Brussels

SATURDAY 21 MORNING

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09:00-12:15: **Cytology**
Chairpersons: B. Weynand (Leuven), C. Bourgain (Bonheiden)

09:00-10:00: ***The Bethesda system for reporting cervical cytology: patterns and pitfalls in squamous and glandular lesions.***
R. Nayar (Chicago, U.S.A.)

10:00-10:30: ***CINtec PLUS.***
C. Bergeron (Cergy Pontoise, France)

10:30-11:15: ***Coffee break***

Visit of the Augustijnenklooster Library: 20 persons maximum. Please register at the Desk.

11:15-11:45: ***Next generation sequencing on cytological samples.***
G. Troncone (Napoli, Italy)

11:45-12:30: ***Application of immunohistochemistry in cytology specimens.***
F. Schmitt (Porto, Portugal)

Room Augustin

09:00-12:15: **Ethics and economy: costs and benefits in pathology**
Chairpersons: J.P. Bogers (Antwerp), P. Demetter (Brussels)

09:00-09:30: ***Next generation sequencing for personalised cancer treatment: worthwhile at the population level?***
M. Van den Bulcke (Brussels)

09:30-10:00: ***Sentinel node biopsy in patients with cutaneous melanoma: cost-efficient?***
P. Serra-Arbeloa (Pamplona, Spain)

10:00-10:30: ***Democratising data-driven medicine.***
K. Eycken (UZ Leuven)

10:30-11:15: ***Coffee break***

Visit of the Augustijnenklooster Library: 20 persons maximum. Please register at the Desk.

11:15-12:00: ***HPV screening algorithms for the prevention of cervical cancer: weighing benefit, cost and burden.***
H. Berkhof (Amsterdam, The Netherlands)

12:00-12:45: ***Results of EKE COS-Y, program for quality evaluation of computer-assisted cervical screening in Flanders.***
B. Lelie (Knokke)

12:45-13:00: **Closing BWP 2017**
P. Demetter – BWP 2017 President

Room Hippo

13:00-13:45: **General Assembly Belgian Society of Pathology**

Room Kloostergangen

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

EXHIBITION FLOOR

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	C 14	VISIOPHARM
	C 17	HAMAMATSU
	C 18	PROBOSS
	C 19	BIOCARTIS

INVITED LECTURES

INVITED LECTURES

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L 3	C. Stefanato (London, U.K.)	Hair pathology.	
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L 16	T. Gevaert (Leuven)	Belgian checklists for prostate cancer (biopsy and RALP).	
L 17	B. Tombal (Brussels)	Is there a role for the pathologists in guiding the treatment of metastatic prostate cancer?	

INVITED LECTURES

FRIDAY OCTOBER 20

SATURDAY OCTOBER 21

Authors have authorised publication of their presentation.

L 2

THE OLD AND NEW CUTANEOUS MUCINOSES.

Franco Rongioletti
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Mucin or protein-hyaluronic acid complex is a jelly-like, amorphous substance which is a normal component of the dermal connective tissue, playing a major role in maintaining the salt and water balance of the dermis. The most common method used for demonstrating mucin in the skin is the alcian blue technique, with which mucin stains blue. However, when an abnormal amount of mucin accumulates in the skin, then we face a pathological condition called cutaneous mucinosis. The cutaneous mucinoses are a heterogeneous group of disorders which are important not only for their dermal disease, but also for the numerous systemic manifestations. It is important that the clinicians be able accurately to diagnose and differentiate scleromyxedema and localized lichen myxedematosus, acral persistent papular mucinosis, scleredema, reticular erythematous mucinosis, pretibial myxedema of hyperthyroidism. Moreover, new diseases in the setting of cutaneous mucinoses have been recently described including AESOP and nevoid follicular mucinosis. Because of the variability of associated systemic manifestations, some with important morbidity and mortality, accurate diagnosis is essential for awareness and appropriate management and the diagnosis is based mostly upon clinico-pathological correlation.

INVITED LECTURES

L 4

CUTANEOUS METASTASES.

Nicolas de Saint Aubain
Institut Jules Bordet

The discovery of skin metastases is relatively rare in clinical practice. The incidence of cutaneous metastases is difficult to evaluate; it has been estimated between 0.7 and 10% of patients with internal malignancies.

The relative frequencies of cutaneous metastases tend to correlate with the frequency of different types of primary cancer in each sex. Lung carcinoma in men, breast carcinoma in women, gastrointestinal carcinomas and melanoma in both sexes are the most common origins of skin metastases.

In the majority of cases, metastases develop after the discovery of the primary neoplasm and do not cause diagnostic problems. However, in up to 37% in males, 6% in females, they appear before the primary neoplasm. The most common sources of precocious metastases are the lung and kidney in males, kidney and ovary in females.

In children, cutaneous metastases represent the first manifestation of the tumor in up to 84% of cases. The most common primary neoplasms include leukemia/lymphoma, rhabdomyosarcoma and neuroblastoma.

Skin metastases can affect any site, but tend to appear in an area close to the primary tumor. Overall, there is a predilection for the head and neck area in males (75%) and the anterior chest wall and the abdomen in females (75%). Some tumors tend to metastasize in specific sites, so the site of the metastasis can guide the search for the primary tumor.

Lung carcinoma tend to metastasize in the anterior chest or the head/neck area. Posterior chest wall metastases are rare but often related to lung small cell carcinoma. Breast carcinoma tend to affect the anterior chest and abdominal wall. Renal cell carcinoma has a predilection for the face and scalp. Head/neck squamous cell carcinomas usually metastasize in the face or neck. Digestive tract carcinomas affect the abdominal wall and pelvis. Umbilical metastases, known as Sister Mary Joseph's nodules, are mostly associated with digestive tract carcinomas: colon, pancreas, or ovarian, breast carcinomas.

Clinical presentation is highly variable. Most skin metastases present as a single nodule, which can be ulcerated, or a cluster of nodules. Most lesions are asymptomatic. Renal cell carcinoma metastases tend to present as reddish nodules which can be confused with hemangiomas. Lymphatic occlusion by neoplastic cells may lead to lymphedema and carcinoma "en cuirasse" (diffuse thickening of the chest wall). "Erysipeloid" or "inflammatory" carcinoma is often seen on the chest in association with breast carcinoma and can be difficult to distinguish from mastitis. "Zosteriform" carcinoma present as multiple vesicles or papules affecting dermatomes, simulating herpes zoster infection. Acral metastases are rare but often painful and can be mistaken for an infectious process. Alopecia neoplastica is mostly associated with breast cancer (85%).

The prognosis of skin metastases is poor, with an estimated mean survival of 6 months.

Table 1. Most common primary tumors according to the site of the metastases

- Scalp: breast, lung, kidney
- Face: lung, kidney, head/neck SCC
- Neck: head/neck SCC
- Anterior chest wall: breast, lung NSCLC
- Posterior chest wall: rare, lung SCLC
- Abdomen: breast, colon, lung, ovary, stomach
- Umbilicus: colon, ovary, stomach, pancreas, breast
- Limbs: breast, lung, kidney, colon

A first immunohistochemical battery for metastatic carcinoma usually include high-molecular weight keratins (SCC, urothelial carcinoma) and CK7/CK20, and will be followed by a second battery of more or less organ-specific markers, depending on the clinical presentation, morphological features and the CK7/CK20 profile: p63, p40 (SCC), TTF1 (lung, thyroid), Napsin A (lung), GATA3 (breast, urothelial carcinoma…), PAX8 (kidney, thyroid, ovarian serous carcinoma…), CDX2, villin (colon), PSA, NKX3.1 (prostate), RCC (kidney), Arginase, HepPar1 (liver), mammaglobin, GCDFP15 (breast), SOX10 (triple negative breast carcinoma), WT1 (ovary,

mesothelioma)… It must be kept in mind that none of this markers is entirely specific and their interpretation always require clinic-pathological correlation. For example, hormone receptors and GATA3 are not uncommonly expressed in lung carcinoma, CDX2 is expressed in mucinous carcinoma of diverse sites, TTF1 is expressed in 30-50% of extrapulmonary small cell carcinomas.

In children, most skin metastases are related to lymphoma/leukemia, rhabdomyosarcoma or neuroblastoma. The immunohistochemical panel should include myogenin (RMS), NSE (NB) and haematological markers. LCA and B/T markers are not sufficient to rule out lymphoma/leukemia and the panel should be extended with markers such as CD30, ALK (anaplastic large cell lymphoma), TdT (lymphoblastic lymphoma), CD4, CD8, CD43, MPO, CD34 (leukemia)…

Table 2. CK7/CK20 expression

CK7-, CK20+	colon, merkel cell carcinoma
CK7+, CK20+	bladder, upper GI, pancreas
CK7-,CK20-	liver, prostate, clear cell RCC
CK7+,CK20-	everything else

The differential diagnosis between primary cutaneous carcinoma and skin metastasis can be extremely difficult and often requires strict clinico-pathological correlation. Specifically, there is significant immunohistochemical overlap between skin and breast carcinomas (both express CK7, CGDFP15, ER/PR…). Clear cell hidradenoma and sebaceous neoplasms can be mistaken for metastatic clear cell carcinoma of the kidney. Ductal carcinomas can be difficult to distinguish from CK7+/CK20- metastases. Primary mucinous carcinoma of the skin may be indistinguishable morphologically from colon (CK20+/CDX2+) or breast metastatic carcinoma. Signet-ring carcinoma of the eyelid is a rare primary cutaneous tumor which can be mistaken for metastatic lobular carcinoma of the breast or gastric signet-ring carcinoma. Secretory carcinoma is a recent entity exhibiting analogous features to breast/ salivary glands secretory carcinoma, including a ETV6-NTRK3 gene fusion.

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L 5

MOLECULAR ASPECTS OF THE LATEST WHO CLASSIFICATION OF LYMPHOMAS.

A. Dendooven, Antwerp

Over the past few years, we have learned a lot about the molecular make-up of haematological malignancies. Advances in our understanding have been driven largely by the application of deep sequencing techniques on (pre)tumoral tissues.

As for lymphomas, this progress has led to an updated version of the WHO classification, leading us to use now WHO 2016 instead of WHO 2008 for categorizing lymphoid malignancies.

In my presentation, I will go through the major changes and adaptations in the current WHO, published in Blood 2016;127:2375-2390; emphasizing what a general pathologist should know and when specific molecular testing should be sought for.

INVITED LECTURES

L 7

CAN MOLECULAR BIOLOGY IMPROVE THE DIAGNOSIS OF THYROID NODULES?

Nicky D'Haene
Department of Pathology, Erasme University Hospital, Université Libre de Bruxelles (ULB), Brussels, Belgium

Abstract

The assessment of thyroid nodules is a common clinical problem. The frequency of thyroid nodules detected by ultrasound in the adult population is almost 67%. On the other hand, thyroid cancer is rare and is found in about 5% of the thyroid nodules. As the majority of thyroid nodules are benign, the challenge of the physicians who manage patients with thyroid nodules is to efficiently stratify patients according to their risk of malignancy in order to identify the best follow-up and therapeutic options. Fine-needle aspiration (FNA) followed by cytological assessment, a non-invasive procedure, has become the predominant method used for the primary diagnosis of benign and malignant thyroid nodules, resulting in the categorisation of patients as operative or non-operative candidates. The diagnosis is based solely on cell aspirates (cytological diagnosis). However, FNA has intrinsic limitations in distinguishing between benign and malignant follicular lesions. More particularly, management of patients with indeterminate cytology still remains problematic. Every proposed thyroid cytological classification is associated with one or more indeterminate categories with a global incidence varying from 10 to 26%. Currently, as this category is associated with a 14% to 48% incidence of malignancy, patients are referred for surgery. This approach introduces a major health-care problem since it leads to unnecessary surgery for patients with a benign lesion.

Efforts to improve the management of patients with indeterminate cytology have focused on identifying additional clinical and US data that efficiently predict malignancy. The recent progress in understanding the molecular pathogenesis of thyroid cancer has identified different potential molecular markers. This has mainly resulted from the identification of molecular alterations of signalling pathways, such as the RAS-RAF-MEK-MAPK pathway. A prominent example is the T1799A transverse point mutation of BRAF, which results in the expression of the BRAF-V600E mutant protein and causes constitutive activation of this serine/threonine kinase. BRAF V600E mutation occurs in approximately 45% of papillary carcinomas and is associated with a poor outcome. Second in prevalence to BRAF mutations in thyroid cancer are RAS mutations (KRAS, NRAS and HRAS). As RAS mutations occur commonly in follicular adenomas, the association between RAS mutation and malignant potential is still unresolved. This could suggest that activated RAS may have a role in early follicular thyroid cell tumorigenesis. However, additional genetic alterations other than RAS mutation are apparently required to transform follicular adenoma into thyroid cancer. Gene translocations resulting in oncogenic gene fusions are also present in thyroid cancer and are best exemplified by RET–PTC or PAX8–PPARG. There are more than 10 types of RET–PTC fusion genes determined by the type of partner genes.

All these genetic alterations in thyroid cancer prompted the search for somatic mutations in material obtained by FNA so as to increase the diagnostic accuracy of cytology. Several prospective studies have shown that testing for mutation in BRAF and RAS genes and the detection of gene rearrangements are feasible on FNA material and provide helpful diagnostic information. These studies have led to the development of commercially available molecular tests that include a panel of genetic alterations commonly seen in thyroid cancer. However, as stated by the American Thyroid Association's guidelines long-term outcome data on companion use of molecular marker status to guide therapeutic decision-making is currently lacking. Nevertheless, the American Thyroid Association's management guidelines now recommend that molecular testing may be used to supplement malignancy risk assessment data for nodules with indeterminate FNA cytology. However, these molecular tests are not yet widely applied in daily practice in Europe. This can be explained by the fact that the number of markers to test is high and that the sequential analysis of so many markers is too expensive and time-consuming.

Recently, a new technology, next generation sequencing (NGS), has emerged for gene panel sequencing. This technology enables the simultaneous sequencing of millions of short DNA sequences and offers benefits such as lower costs, increased workflow speed and enhanced sensitivity in mutation detection. Different studies showed that NGS can be successfully applied on cytological material. However, only a few teams report the use of NGS for thyroid FNA.

A proprietary thyroid-specific gene panel for NGS (ThyroSeq) has been developed providing simultaneous sequencing for mutation detection in 14 thyroid-cancer-related genes and for 42 types of gene fusions occurring in thyroid cancer. ThyroSeq has been tested for its accuracy and performance in diagnosing malignancy in thyroid nodules with indeterminate cytopathology in two single-center studies (Nikiforov 2014; Nikiforov 2015). The reported performance of this panel is quite similar in the two studies with a 90 - 90.9% sensitivity, 92.1 - 93% specificity, 96- 97.2% NPV and 76.9 - 83% PPV.

As an alternative, we have used the Ion AmpliSeq Cancer Hotspot Panel (Thermo Fisher Scientific Inc), a commercially available primer pool for sequencing generic cancer genes, including the BRAF, NRAS, HRAS, KRAS, and RET genes. We retrospectively analyzed 34 indeterminate FNA samples from patients on whom surgical resection was performed; histological diagnosis was considered as the gold standard (Le Mercier, 2015). DNA from these 34 samples was obtained either from cell blocks of from Diff-Quick stained smears and subjected to targeted NGS with the Ampliseq Cancer Hotspot Panel. All samples were successfully sequenced. Mutations in BRAF, NRAS and KRAS were detected in 7 FNA samples. The presence of a mutation in one of these genes was a strong indicator of cancer, since 5 (71%) patients with a mutation positive FNA had malignant diagnosis after surgery. Moreover, in the same experiment NGS allowed us to detect rare or low frequency mutations in other genes such as TP53, which can have a prognostic impact.

The detection in these specimens of different mutations known to be involved in thyroid carcinoma biology can improve sensitivity of the diagnosis in thyroid FNA samples, although this approach needs further improvement. Indeed, only point mutations were evaluated with the Ion AmpliSeq Cancer Hotspot Panel. The combination with the detection of gene fusions could improve the sensitivity of molecular screening.

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L 8

MOLECULAR PATHOLOGY AND TESTING OF BRAIN TUMOURS.

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The 2016 WHO classification of central nervous system tumors for the first time uses molecular parameters in addition to histology to define many tumor entities.

In this talk, I will give an overview on molecular testing required for the diagnosis of brain tumors. Specifically, the diagnostic approach to glioblastoma (IDH-wildtype and mutant); diffuse midline glioma H3 K27M–mutant; oligodendroglioma IDH-mutated 1p/19q-codeleted, RELA fusion–positive ependymoma and medulloblastoma (WNT-activated and SHH-activated) will be presented. Furthermore, the role of MGMT promotor methylation status as a prognostic marker will be discussed. Special emphasis will be given on problems and practical solutions in a surgical neuropathology lab.

L 9

PD-L1 IN LUNG CANCER.

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Since over a decade the usual pulomonary pathology approach was changed from diagnosis only to diagnosis and prediction. The biomarker for EGFR tyrosine kinases turned out to be EGFR mutation analysis. Subsequently, ALK FISH analysis was the biomarker for ALK inhibitor treatment, which may be replaced by ALK immunohistochemistry with 5A4 or D5F3.

Beyond these first simple predictive biomarker analyses additional genes ROS1, RET, MET, and PD-L1 came into play, with more on the horizon like e.g. NTRK1 and NRG1.

One aspect that needs special attention is how to perform clinical validation for PD-L1.

The most important factor on a global scale influencing the accuracy of PD-L1 IHC as a predictive biomarker of clinical benefit to PD-(L)1 checkpoint inhibition is its proper clinical validation. This needs to be performed on a technical level involving the appropriate samples and the clinical established threshold. Basically three strategies exist. Firstly, by using the samples of the clinical phase III studies with annotated outcome and examine the performance of the PD-L1 assay. As the availability of tumor tissue (mainly biopsies) is limited, extensive testing is prohibited.

The second approach is an indirect approach where the starting point is the PD-L1 assay used in the phase III study, because of its validated threshold with associated clinical relevance. The assumption with this approach is that the commercial assay is robust in time.

For selection of the samples quantitative IHC learns that those samples with a high epitope concentration will reach the maximum level of staining and are likely to be positive in any PD-L1 assay.[1] This notion may also hold for placenta as an external positive control. Likewise, samples with a very low epitope concentration will be negative with any PD-L1 assay. For adequate comparison of different PD-L1 IHC assays these samples will also not be informative, as these will likely have the same outcome in the different assays. In contrast, samples with a PD-L1 epitope concentration close to the threshold of positivity of the clinical validated PD-L1 assay are specifically suited for comparison, further called critical samples. All other PD-L1 assays, laboratory developed tests as well commercial tests with other antibodies, may be checked on critical samples for an equivalent outcome compared to the clinical validated test, see figure 1. Ideally, a mixture of critical and non-critical samples is used, also for proficiency testing in external quality assessment schemes. If discordancy occurs in the non-critical samples in the alternative PD-L1 assay with the validated commercial assay (associated with a specific drug e.g. 22C3 with pembrolizumab), the alternative assay is clearly not validated. Comparing a few serial sections of sufficient (n= ~10) [2] but limited number of critical samples on both sides of the threshold stained with different assays is adequate. Obtaining equal staining patterns between the validated and the alternative test, is sufficient for validation.

The third approach is the statistically driven comparison of > 800 samples according to the FDA with the requirement of 85% concordancy.[3] This approach is very expensive (1 million US dollar on a commercial basis). The question arises if the fraction of critical samples is less than 10%, it is not excluded that comparison of almost ANY of the assays will reach an 85% concordancy once a high enough number of tumor samples (800?) is compared, without having the same PD-L1 staining pattern on critical samples.

The published studies on comparison of PD-L1 assays have been focused on technical validation, but non of them so far implemented clinical validation[4–6].

The first PD-L1 external quality assessment by NordiQC revealed that according to their standards the pass rate for CE IVD /FDA approved assays and laboratory developed test was 80% and 20%, respectively.[7] This important effort Indicates that

commercial tests were adequate, but need also attention to maintain sufficient quality and the laboratory developed tests should not be used unless carefully validated. A French study reached more or less the same conclusion.[8]

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L 11

INTRAHEPATIC VASCULAR DISEASES: REVISITING AND EVOLVING ENTITIES.

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Liver functions are tightly dependent on adequate circulation. The liver receives 25% of cardiac output and is responsible 20% of total body oxygen consumption. Microcirculation at level of hepatic lobules is the principle site of metabolic exchanges between hepatocytes and blood and for hepatic blood flow regulation. It is constituted of two inflows system intimate related (portal vein and hepatic artery), a capillary system (sinusoids) and one outflow system (hepatic vein). The sinusoids is lined by endothelium, boarded by space of Disse (minimal connective network of reticular fibres (collagen type III) and hepatic stellated cells). It is the site of portal venous and arterial blood mixture (at a volume ratio of 7:3) and most of important liver functions (gas exchange, nutrients absorption, scavenging and detoxification, immune reactions, vitamin A storage). The metabolic exchanges between hepatocytes and blood are facilitated (low sinusoidal pressure, lack of basement membrane, fenestrated endothelial cells, microvilli on sinusoidal membranes of hepatocytes that increase surface area). At microscope, sinusoids appear as slit like spaces when cut longitudinally and circular when cut in cross-section. They have variable orientation and size according to localization within lobule, periportal sinusoids being more torturous. There average diameter range between 4 to 10µm, and may distend to 15-20µm, probably due to physiologic difference in intrasinusoidal pressure. Sinusoids usually appear empty or may contain few red blood cells or a sprinkling of inflammatory cells or rarely, megakaryocyte; they outflow trough venular outlet. Recent concept of normal microcirculation anatomy (concept of lobular vascular septa), nomenclature for liver histology and quantitative references standard of normal liver histology on normal adult liver biopsy are references which helps standardisation.

Intra-hepatic vascular lesions may touch the various components of intrahepatic circulatory system and can have different clinical and pathological consequences. A common feature is the risk to develop portal hypertension (PHT) with correlated increase morbidity and mortality.

Main cause of intrahepatic PHT is cirrhosis (regardless of its aetiology). The block is mainly at sinusoidal level associated to specific vascular lesions, such as vascular shunts, neoangiogenesis, capillarization of sinusoids, portal tract venous thrombosis, and parenchymal extinction.

Other cause of intrahepatic PHT is grouped until currently under the term idiopathic non-cirrhotic portal hypertension (INCPH). Its formal diagnosis is presently based on the following criteria: 1) presence of unequivocal signs of portal hypertension, 2) absence of cirrhosis, advanced fibrosis or other causes of chronic liver diseases, and 3) absence of thrombosis of the hepatic veins or of the portal vein. It recovers very various aetiologies that can be grouped in five major categories: 1) immunological disorders (i.e. association with common variable immunodeficiency syndrome, connective tissue diseases, Crohn's disease, etc.), 2) chronic infections, 3) exposure to medications or toxins (e.g. azathioprine, 6- thioguanine, oxaliplatin), 4) genetic predisposition and 5) prothrombotic conditions (e.g. inherited thrombophilias myeloproliferative neoplasm, antiphospholipid syndrome).

Underestimated in his frequency and of not sufficiently understanding on its pathogenesis, INCPH is today subject to great renewed interest and increased recognition. Undoubtly, INCPH nomenclature is confusing and needs nowadays a reconsideration. In fact today, increasingly number of patients are identifying at a stage where they have no signs of portal hypertension but only mild alterations in liver enzymes, mild or not alterations in liver morphology at imaging studies, but histological findings at liver biopsies and, in some cases, associated conditions such as those described in patients with the full INCPH picture. Although the natural history of this group of "asymptomatic" patients is far from well characterized, it is evident

that a significant number will progress to develop the full portal hypertension picture. A proposal for a new homogeneous nomenclature of a more inclusive entity that would also incorporate pre-clinical asymptomatic forms is today under study and proposed by VALDIG (vascular liver disease group) supported by EASL (European association for the study of liver). Histological lesions associated to INCPH being located in the hepatic microcirculation, this brought to the new terminology "Porto-Sinusoidal Disease (PSD)".

4 major defined pathologic entities are known to be associated to INCPH, namely - sinusoidal obstruction syndrome (SOS), - nodular regenerative hyperplasia (NRH), - obliterative venopathy, and - incomplete septal cirrhosis.

Sinusoidal obstruction syndrome (SOS): this entity was originally known as veno-occlusive disease (VOD), in reference to histologically evident centrilobular vein occlusion (by subendothelial oedema, haemorrhage or fibrosis), mandatory for diagnosis. Experimental studies have considerably clarified its pathogenesis and have shown that centrilobular vein involvement is not essential in the development of SOS. The main injury occurs at the level of hepatic sinusoids. This led today to general acceptance of SOS term in preference to VOD. Diagnosis is currently based on sinusoidal lesions, independently of hepatic venous lesions. Centrilobular vein occlusion is thus today no more a mandatory feature for diagnosis on liver biopsy and is more a complication and sign of severe disease. SOS is histologically defined by a rupture of the sinusoid integrity with various degree of centrilobular sinusoidal dilatation, congestion, and haemorrhage with possibly damage and loss of hepatocytes. Spectrum of lesions associated to SOS are perisinusoidal/ centrolobular fibrosis, nodular regenerative hyperplasia and/or peliosis.

Latency to clinical onset of SOS in human is highly variable and can go from acute form meaning within 1 to 3 weeks of exposure to the medication which can be a single infusion (for ex, in hematopoietic stem cell transplantation in first 20 days with cyclophosphamide, later with other regimens), to subacute and chronic forms present weeks, months or even years after starting (for ex: toxic oil syndrome; azathioprine and 6-thioguanine, symptomatic over time in relation to additional circumstance (ex: oxaliplatine and hepatectomy).

Clinical features in HSCT is the setting where it is most clearly defined. Acute SOS: abdominal pain, weight gain and signs of portal hypertension (ascites, edema, varices). Jaundice generally mild or absent initially; worsen if injury is severe. Subacute and chronic forms: fatigue and abdominal swelling with signs and symptoms of portal hypertension including edema, ascites, varices, hepatic encephalopathy or muscle wasting and weakness. In Oxaliplatin associated SOS, can be observed a longer surgery and hospital stay, decrease accuracy of metastasis detection on post-chemotherapy imaging, infections, increase risk of peroperative hemorrhage, poor liver function reserve and post-operative liver failure after major hepatectomy, portal hypertension and ascites, rare case of death, low tumoral response. Reversibility of SOS and associated lesions is under study; it has recently been estimated to more than 9 months between end of chemotherapy and histological.

Nodular regenerative hyperplasia (NRH) is a transformation of normal hepatic parenchyma in small regenerative nodules, mostly 1-3 mm (rarely larger), often paler than normal parenchyma. Compared to cirrhotic nodules, they are less well defined nodules, since not limited by fibrosis but sometime by congestion. Normal size of liver or hepatomegaly with surface smooth or only partially irregular. Nodular transformation: confined to one portion of liver or diffuse. Accurate diagnosis made by histology. Distortion of lobular architecture by nodules of hyperplastic hepatocytes centred by portal tract Surrounded by compressed atrophic cell plates and central veins and little to no fibrosis Occasional curvilinear sinusoidal dilatation in areas of atrophy No inflammation Nodular transformation in NRH: related to alterations in intra-hepatic blood flow = "atrophy-hypertrophy complex". Imaging methods poor sensitivity and specificity for NRH Among patients referred for unexplained liver enzyme abnormalities (n=750) , 15% had NRH , age 53 years old. In H&E needle biopsy, changes subtle. Therefore, any "normal" liver biopsy (particularly from patients with INCPH) should be investigated using reticulin stains and possibly with IHC for CK7. Very little data on long term prognosis and outcome and reversibility once presumed cause is removed. Prognosis usually better than other chronic liver diseases and is related to the complications of PH and the severity of associated diseases, if present. Rare cases of HCC have been reported. Histological

regression of NRH with normalization of liver enzymes in four patients after withdrawal of AZA, after being used for an average of 64 months.

Obliterative venopathy. Whereas larger portal vein may be widely patent, portal vein structures in small radicals may be absent or occluded. Also known as Hepato-portal sclerosis.

Incomplete septal cirrhosis. This entity is characterized by the presence of - Thin perforated fibrous septa; - Isolated collagen bundles within parenchyma;- Hepatocytes within septa;- Periportal fibrous spicules ;- Isolated portal spaces and hectic veins. The debate is open to know is it correspond to a cirrhosis in reversion or the end point evolution of an obliterative venopathy.

Finally, several other individual histological features are **suggestive** of INCPH

- Portal tract
  Abnormally positioned portal veins
  Herniating in periportal area (Paraportal shunting vessels)
  Artery : multiplication, hyperplasia
  Abortive portal tracts
- Lobular architecture disruption
  Loss of regular alternance between PT/CV
  Portal tract crowding
  Multiplication/ dilatation of draining vessels
  Portal tract/ central vein approximation
- Sinusoids
  focal sinusoidal dilatation
  Perisinusoidal fibrosis

In conclusion, vascular diseases of the liver represent a significant health problem in the field of liver disease. A common characteristic shared by many such diseases is their propensity to cause portal hypertension together with increased morbidity and mortality. Pathogenesis not fully understood, and probably involves different mechanisms. Entities have to be redefined clinically and pathologically for further studies.

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INVITED LECTURES

L 12

DRUG-INDUCED GASTROINTESTINAL DISEASE.

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Diagnosis of drug-induced injury in the gastrointestinal tract is difficult. Some compounds are associated with characteristic patterns of injury, however, many are not. Although numerous drugs are implicated, one of the commonest offending agents are non-steroidal anti-inflammatory drugs (NSAIDS). Mycophenolic acid & angiotensin II receptor blockers (ARBS) have also recently been highlighted in the literature. Patterns of injury generally are not specific & mimic many common gastrointestinal conditions including coeliac disease, graft versus host disease (GVHD), neoplasia and inflammatory bowel disease (IBD). Subclassification of the injury patterns into five categories is a useful exercise. 1. Villous atrophy. 2. Apoptotic/erosive. 3. Abnormal mitoses. 4. Ulcerative. 5. Crystal deposition. Since the gut has a limited set of response patterns to injury: clinical correlation is always important (when little or no clinical information is usually provided). Drug-induced injury should always be considered if an atypical "itis" is observed. Histological pointers include: 'Apoptotic -itis', 'Withering of colonic crypts', 'eosinophilic crypt abscesses' and 'Ring mitoses'. Checkpoint inhibitors, including PDL1 & PD1 inhibitors can also induce a pan-enteritis & a severe colitis. These agents can also exacerbate existing IBD. Novel therapeutic agents including 'biologics' used in the treatment of IBD & other auto-immune conditions may also damage the gastrointestinal tract.

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L 14

BIOMARKERS IN PROSTATE CANCER.

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The Gleason score is one of the most important parameters for clinical decision-making in prostate cancer patients. While men with Gleason score (GS) 6 are often eligible for active surveillance, patients with GS 3+4=7 and higher are generally treated for their disease. However, there is discussion about whether men with biopsy GS 3+4=7 need to be treated in all cases. While pathologic distinction of GS 6 and 3+4=7 is essential for clinical decision-making, inter-observer variability in grading is considerable (1). Surveillance is therefore increasingly considered in men with biopsy GS 3+4=7 (2, 3). Both pathologic and molecular biomarkers can strongly support therapeutic decision-making in GS 3+4=7 prostate cancer patients.

GS 7 is a heterogeneous group of tumors consisting of well-delineated Gleason pattern (GP) 3 glands and any amount of GP4 structures. Pathologically, at least four different growth patterns are regarded as GP4, namely ill-formed, fused, cribriform and glomeruloid (4). While these growth patterns are not routinely specified in pathology reports, it has become clear that they are clinically and biologically different. GS 7 patients with cribriform growth at radical prostatectomy suffer from biochemical recurrence and metastasis significantly more often those without this pattern (5-7). Patients with biopsy GS 3+4=7 with cribriform growth show more frequent biochemical recurrence after operation and have shorter disease-specific survival (8, 9). On the other hand, those GS 3+4=7 men without cribriform pattern show similar recurrence and survival rates as men with biopsy GS 6. The clinical significance of cribriform pattern is independent of its quantity; even GS 3+4=7 tumors with low amounts of cribriform growth show aggressive behavior (7, 8). Putatively, cribriform growth is a morphologic substrate of increased genomic instability which might be rationale for its behavior (10). On the other hand, the recognition and classification of cribriform and glomeruloid as GP4 is far more reproducible than identification of ill-formed and fused growth patterns (1, 11). Recent three-dimensional imaging reveals that GP 3 glands form a morphologic continuum with ill-formed and fused GP4. The significant inter-observer variability and good outcome of GS 3+4=7 with ill-formed and fused growth patterns might raise the question on legitimacy of labelling them as GP 4.

Apart from distinguishing growth patterns, GP4 quantity adds information to stratification of GS 3+4=7. In a very large radical prostatectomy study, Sauter and colleagues have shown that biochemical recurrence is incremental with increasing GP4 (12). In biopsy specimen, GP4 quantity is associated with adverse pathologic parameters at radical prostatectomy. GS 3+4=7 with 1-25% GP4 show more indolent features than those with 25-50% GP4. This is another rationale for offering active surveillance to GS 3+4=7 (2, 3). Therefore, the WHO 2016 blue fascicle advices to include GP4 quantity in pathology reports of prostate cancer (13).

The third important pathologic biomarker in GS 3+4=7 prostate cancer is presence of intraductal carcinoma (IDC) which is characterized by enlarged pre-existent glands filled with a cribriform or solid proliferation of malignant cells or tufts of highly atypical epithelial cells. IDC at biopsy is associated with adverse pathologic features at radical prostatectomy (14, 15). It is hypothesized that IDC represents a retrograde expansion of aggressive invasive carcinoma into pre-existent ducts, albeit primary pre-invasive development of IDC might also occur. On a molecular level IDC is associated with PTEN loss, which could be used as a marker for distinction with high-grade PIN in borderline cases (16, 17).

Cribriform growth pattern distinction, GP4 quantity and presence of IDC all provide additional information for risk stratification of GS 3+4=7. Although these pathologic parameters have mostly been studied separately, the question raises whether they are all independent or not. Recently, it was shown that invasive cribriform growth is associated higher amounts of GP4 at biopsy demonstrating a close link between both pathologic parameters (18).

On the other hand, distinction of invasive cribriform growth and cribriform IDC might be difficult, if not impossible without the use of basal cell immunohistochemistry.

While many studies have described potential clinical value of immunohistochemical and molecular markers in prostate cancer patients, a limited number of commercial tests are currently available for risk stratification of GS 3+4=7 (see review) (19). Although these validated assays could support clinicians in decision-making, their use in daily practice is still limited in Europe. These commercial tests have been developed and validated in study populations using 'standard' clinicopathologic parameters such as GS, PSA value and tumor volume surrogates as covariates. It is of interest to study the performance of respective tests in the light of 'novel' pathologic risk parameters such as invasive cribriform growth, GP4 quantity and IDC. For future studies and definite risk stratification it is important to consider respective pathologic and molecular parameters, and investigate their mutual relation.

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L 18

HPV-RELATED TUMOURS IN THE 2017 WHO CLASSIFICATION AND DIFFERENTIAL DIAGNOSIS.

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High-risk HPV related oropharyngeal carcinoma is an epidemiologically, pathologically and clinically distinct form of head and neck squamous cell carcinoma. Since the 2017 edition of the WHO Classification of Head and Neck tumours, the tumours of the oropharynx (base of tongue, tonsils, and adenoids) are discussed separately from the tumours of the oral cavity and the mobile tongue. The squamous cell tumours of the oropharynx are further classified as HPV-positive or HPV negative.

HPV related oropharyngeal carcinoma: an epidemic sexually transmitted disease

The prevalence and incidence of oral high-risk HPV detections is associated with an increased number of sex partners during lifetime and with practising oral sex. Open mouth kissing has also been suggested as a risk factor. At any given point in time, approximately 7% of the population has a prevalent oral HPV infection, but the majority of the infections is cleared. However, the infections can persist and lead to the development of oropharyngeal squamous cell carcinoma (OPSCC), especially in men and more so if they also smoke.

The typical patient with HPV+ OPSCC is a white male, 50-60 years of age, a history of mild smoking and an above average of sexual partner.

The incidence of HPV related OPSCC is rising mostly in economically more developed countries. In the US a 225%, increase in HPV related OPSCC has been noticed between 1984 en 2004, whereas HPV negative OPSCC has decreased by 50%. Approximately 70% of the OPSCC in the US are HPV related and the incidence of HPV+ OPSCC has surpassed this of cervical carcinoma.

In Belgium OPSCC is the most prevalent head and neck cancer. Twenty-five percent of the OPSCC in Flanders are related to HPV.

p16 Immunoreactivity as surrogate marker for transcriptional active HPV-related OPSCC

HPV testing can be done by DNA testing, mRNA testing for the oncogenic proteins, or in situ hybridization. However, a practical and reproducible method is immunohistochemistry for p16. Overexpression of p16 indicates the presence of transcriptionally active HPV. Binding of the viral oncoprotein E7 with the Rb protein and its subsequent degradation result in activation of the transcription factor E2F, which induces the expression of S-phase genes. As a feedback mechanism, the p16 protein expression is upregulated.

p16 Positivity is defined as nuclear and cytoplasmic staining in > 70 % of the tumour cells. The use of p16 as surrogate marker for HPV in head and neck tumours is exclusively valid for OPSCC.

HPV/p16+ OPSCC has a better prognosis than HPV/p16 negative OPSCC

Patients with HPV+ OPSCC have a 2 to 5 times lower risk for overall and disease specific mortality. Transcriptionally active HPV in OPSCC is an independent predictive factor of better patient survival. Since the eight edition of the TNM classification p16 positive and p16 negative OPSCC are classified differently for the N status and p16+ OPSCC are downgraded.

In consequence, all new OPSCC primary tumours should be tested for p16 or HPV.

HPV/p16+ OPSCC histological characteristics

Non-keratinizing SCC of the oropharynx is almost diagnostic for transcriptionally active high risk HPV associated OPSCC. Nighty-nine percent of the non-keratinizing OPSCC are p16 +. The non-keratinising

phenotype includes basaloid and lymphoepthelial-like SCC. However, HPV/p16+ carcinomas can be keratinising, non-keratinising with maturation, papillary, ciliated or can harbour a small cell neuro-endocrine carcinoma.

Grading is not applicable for HPV/p16+ OPSCC. These carcinomas are presumed to originate from the reticulate crypt epithelial, which is already a morphologically immature epithelium, and transcriptionally active HPV in OPSCC is an independent prognostic factor for survival. The only exceptions are OPSCC with anaplasia, multinucleation and OPSCC with a small cell neuro-endocrine component. These features should be reported whenever present.

Though in theory, crypt dysplasia can be a precursor lesion in HPV/p16+ OPSCC there is no use for the terms dysplasia or carcinoma in situ when dealing with HPV/p16+ OPSCC. The crypt epithelium is a reticulated epithelium with a discontinuous basement membrane and capillaries running between the epithelial cells. As such, the crypt epithelium is a poor barrier for the spread of carcinoma.

HPV/p16+ OPSCC and neck metastasis

In line with the crypt epithelium being a poor barrier to metastasis, 80% of the patients with HPV/p16+ OPSCC present with lymph node metastasis at the time of diagnosis. Metastasis are often, solitary, large and cystic. Primary presentation with a neck metastasis is not uncommon. Seventy-five percent of the primary neck node metastasis are squamous cell carcinomas and in 90% of these cases the primary tumour is subsequently found in the palatine tonsils or the base of the crypts.

It is advised that all neck node metastasis of an unknown primary should be tested for HPV of p16. p16 Immunohistochemistry as stand-alone test is only to be used for metastasis in lymph nodes in level II or III and only for tumours with non-keratinizing morphology.

Not finding the primary tumour in a patient who has been diagnosed with a p16+ non-keratinizing SCC in a lymph node in level II or III, does not preclude a primary in the tonsils, but indicates an occult tumour hidden somewhere in the crypts.

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INVITED LECTURES

L 19

TUMOUR OF THE NASAL CAVITY AND PARANASAL SINUSES – THE 4 TH WHO CLASSIFICATION.

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Three new entities have been included in the chapter on tumours of the nasal cavity, paranasal sinuses and skull base in the 4th edition of the WHO Head and Neck tumour classification[1], compared with the previous edition[2]: NUT carcinoma, seromucinous hamartoma and biphenotypic sinonasal sarcoma.

In the group of "small round cell tumours" of the sinonasal tract NUT carcinoma is a new entity first described as a mediastinal tumour and also known as NUT midline carcinoma. It is a cytokeratin positive tumour often with squamous differentiation characterised by the presence of nuclear protein in testis gene (NUTM1) rearrangement.

In the group of respiratory epithelial lesions, the entity "seromucinous hamartoma" has been separated from the already existing "respiratory epithelial adenomatoid hamartoma", where previously both lesions were considered synonymously. It is a benign polypous lesion involving the sinonasal tract with an important number of seromucinous glands.

The malignant soft tissue tumours harbour a new entity called biphenotypic sinonasal sarcoma. This tumour affects predominantly middle-aged females (mean 52 years). It is a low-grade spindle sarcoma most frequently characterized by a PAX3-MAML3 gene fusion. Besides variable staining with S100 the tumour is also positive for actin and calponin. The tumour has to be differentiated from cellular schwannoma, malignant peripheral nerve sheath tumour, solitary fibrous tumour, and synovial sarcoma.

Also in the sinonasal tract it is important to make a distinction between HPV-positive and HPV-negative squamous cell carcinoma similarly to those in other head and neck sites.

In the group of the small sinonasal undifferentiated carcinoma a subset of undifferentiated carcinomas with rhabdoid features and a lack of SMARCB (INI1) protein has been reported as an emerging entity as in the brain[3].

In the non-intestinal-type adenocarcinoma of the sinonasal tract, a renal cell-like carcinoma is a new emerging entity. These tumours express CAIX and CD10 but do not express PAX8.[4]

The classification of the other entities of tumours involving the sinonasal tract globally remain unchanged, although much new molecular biological information is included in the text.

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L 20

ODONTOGENIC AND MAXILLOFACIAL BONE TUMOURS.

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Neoplasms of the gnathic bones are rare and divided in benign and malignant.

The benign group, more common than the malignant group, comprises
- epithelial odontogenic tumors,
- mixed epithelial and mesenchymal odontogenic tumors,
- mesenchymal odontogenic tumors,
- odontogenic inflammatory cysts,
- odontogenic and non-odontogenic developmental cysts,
- bone and cartilage tumors,
- fibro-osseous and osteochondromatous lesions,
- giant cell lesions and simple bone cysts and
- hematolymphoid tumours.

The malignant group includes

- odontogenic carcinomas, odontogenic carcinosarcomas,
- odontogenic sarcomas,
- chondrosarcoma,
- osteosarcoma and
- mesenchymal chondrosarcoma.

The focus will be laid on the more common benign lesions and also the malignant tumors, including new entities. Histopathological characteristics and genetics (if known) and differential diagnoses will be discussed. Hematolymphoid tumors will be excluded.

INVITED LECTURES

L 21

TUMORS OF THE SALIVARY GLANDS – THE 4TH WHO CLASSIFICATION.

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The salivary gland tumor chapter in the 4th edition of the WHO Head and Neck tumor classification1 represents updates and modifications attained since the publication of the 3rd edition (2005)2. Proposed new entities were carefully deliberated based on evidence for proven and validated pathologic characteristics, differential diagnostic relevance and, most importantly, the impact on patient management.

Applying these criteria, secretory carcinoma and sclerosing polycystic adenosis were accepted as a new diagnostic entities. In 2010, Skalova and colleagues3 published a series of salivary gland tumors under the designation "mammary analogue secretory carcinoma", noting that their histologic, immunophenotypic and molecular features mirrored those of secretory carcinoma segregated in a prior study from mammary acinic cell carcinomas4. Subsequent studies of this entity have stressed the identification of the ETV6-NTRK3 fusion gene in the majority of tumors and its importance to the diagnosis. It was generally agreed, at this stage, that although identification of gene fusions may, in some instances, provide support to the histologic diagnosis, morphologic assessment remains the primary factor.

Although sclerosing polycystic adenosis was described long before the 3rd edition of the salivary gland section, it is now officially accepted as a new entry and represents a major entity in the new "Other epithelial lesions" category in the 4th edition. Sclerosing polycystic adenosis got this name for its resemblance to fibrocystic change and sclerosing adenosis of the breast. The age range is broad (typically 4th decade), with a slight female predilection. The vast majority are parotid lesions.

Polymorphous low-grade adenocarcinoma raised a lot of discussion in this update of the WHO classification for salivary gland tumors, at first about either keeping or deleting the qualifier 'low grade' and secondly about the status of a related lesion, the so-called 'cribriform adenocarcinoma of minor salivary gland origin.

Replacing the name polymorphous low grade adenocarcinoma by polymorphous adenocarcinoma was thought to be justified by the reported aggressive nature of a subset of these tumors and the consideration that such a qualifier may exclude patients with advanced disease from clinical therapeutic trials for aggressive salivary cancer.

Whether its unique location at the base of the tongue and the reported more aggressive behavior for "cribriform adenocarcinoma of minor salivary gland origin" justified a recognition of this lesion as an entity separate from polymorphous adenocarcinoma was extensively discussed. However, consensus on its acceptance as a distinct entity was not achieved, since a proportion of pathologists considered the reported examples to represent a variant of polymorphous adenocarcinoma and that similar behavior of polymorphous adenocarcinoma at this location also had been reported5. Accordingly, the entity was considered an "emerging entity" for further consideration in future editions.

A three-tiered grading system was retained for mucoepidermoid carcinoma because of its relevance to prognosis and management. For adenoid cystic carcinoma, the ubiquitous presence of at least two patterns in a given tumor precluded a reliable grading based on distinct morphologic criteria. However, the presence of a solid component and/

or high grade transformation was considered to represent an aggressive tumor. New relative to the prior edition of this chapter is the term high-grade transformation. This has been reported for acinic cell carcinoma, adenoid cystic carcinoma, and epithelial-myoepithelial carcinoma.

An additional notable modification is the requirement to specify the type of carcinoma evolving from pleomorphic adenoma, instead of a generic diagnosis "carcinoma ex-pleomorphic adenoma". Since the majority of malignancies arising in pleomorphic adenoma are salivary duct carcinoma and myoepithelial carcinoma, specifying the malignant component in the diagnosis will eliminate query on the nature of the malignant component, especially for management and biomarker based therapy. When a malignant looking component does not spread beyond the borders of an otherwise benign looking pleomorphic adenoma, the tumor is classified as pleomorphic adenoma with intracapsular carcinoma, the former designations pleomorphic adenoma with atypia or carcinoma in situ in pleomorphic adenoma advocated not to be used anymore. In this way, the concept of stratification by extent of invasion as previously introduced was expanded in this edition, carcinoma ex pleomorphic adenoma being classified as intracapsular, minimally invasive and (widely)-invasive. The current edition is realistic in the approach suggesting that the optimal cut-off to define minimal invasion requires further validation because the initial cut-off of 1.5 mm to delineate minimal invasion has been deemed arbitrary and too restrictive as several studies have justified a 4–6 mm cut-off as still prognostically relevant.6

Lastly, and for their differential diagnostic implications, non-neoplastic cystic and tumor-like conditions were discussed in a separate section, such as nodular oncocytic hyperplasia and intercalated duct hyperplasia.

In keeping with the limited use of lineage and immune-based markers, only the more common markers with documented differential diagnostic relevance were covered. Similarly, the discussion of unique genetic and fusion genes and their relevance was limited for their potential biological and therapeutic implications, with the caveat that their diagnostic implications are currently uncertain. In that context, it is critical that the WHO classification must maintain its universal use as a reference to pathologists who may not have access to advanced ancillary techniques. While acknowledging the importance and the need for lineage, biomarkers and molecular markers to complement the morphologic diagnosis for better classification or subclassification of salivary gland tumors, there is lack of clinical validation and pathologists cannot rely on these markers exclusive of morphologic features. Nonetheless, the future utility of these findings cannot be underestimated.

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INVITED LECTURES

L 22

UTERINE SMOOTH MUSCLE TUMORS, ENDOMETRIAL STROMAL TUMORS AND UTROSCT: STATE OF THE ART AND PERSPECTIVE.

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Uterine mesenchymal tumors encompass smooth muscle tumors (benign leiomyomas [LM] and malignant leiomyosarcomas [LMS]), endometrial stromal tumors (benign endometrial stromal nodules and malignant endometrial stromal sarcomas), undifferentiated uterine sarcomas, adenosarcomas as well as PEComas, inflammatory myofibroblastic tumors, UTROSCT and other rare entities (rhabdomyosarcoma, alveolar soft part sarcoma)(1).

Among the uterine smooth muscle tumors (uSMT), LMs occur in 70% of white women and in >80% of black women by the age of 50 and is 800 times more common than uterine LMS (2). On the contrary, LMS accounts for 1–2% of all uterine cancers and 40% of uterine sarcomas (3).

USMTs (benign LMs or malignant LMS) encompass three histologic subtypes: spindle (conventional type), epithelioid and myxoid. The diagnostic criteria are different among the different subtypes. Spindle cell smooth muscle tumors are differentiated based on the 'three features' (cytological atypia, mitotic count, and tumor cell necrosis) morphological approach, first proposed by investigators from Stanford in 1994 (4). Epithelioid lesions with ≥4 mitoses/10 HPFs with either tumor cell necrosis or at least moderate cytologic atypia are classified as epithelioid LMS. Myxoid leiomyosarcomas can be diagnosed based on the presence of either tumor cell necrosis or marked cytologic atypia; in their absence, ≥2 mitoses/10 HPFs is diagnosed as malignant (5). Irregular or infiltrative tumor borders - prognostic factors for aggressiveness - are also key for this diagnosis (6).

Among the LMs, many subtypes have been described with a wide morphological spectrum, immunohistochemical and genomic characteristics, the most important being, leiomyoma with Bizarre Nuclei/symplastic LM (BN-LM) due to similarity with LMS at morphological and genomic level.

Grossly BN-LM shows white and whorled or yellow and solid, cystic and hemorrhagic degeneration in around 10-32% of the cases (7, 8).

Microscopically, BN-LM shows atypical mono or multinucleated giant cells, nuclear inclusions and chariorrectic nuclei mimicking atypical mitoses. The distribution of "atypical" cells could be focal, multifocal or diffuse, mimicking LMS. A hallmark of a BN-LM is the prominent vasculature made of thick vessels with fibrinoid changes and perivascular inflammatory infiltrate ending in lumen obliteration in an ischemic damage context (7). LM-BN share some morphological, IHC and genomic similarities with LMS that could make the diagnosis very challenging (7-9). It is important to establish the correct diagnosis as the patients are often in their reproductive age. Useful morphological features that differentiate BN-LM and LMS are the absence of atypia and low mitotic activity in the spindle cell component and the patchy distribution of bizarre nuclei (5, 7). Molecular studies have distinguished two groups of BN-LM: 1) associated with FH-deficiency and 2) carrying frequent TP53 and RB1 losses or mutations (8). MED12 mutations are rare in BN-LM (10, 11) and mutually exclusive with FH alterations ((12).

Despite their clinically benign nature, BN-LMs show a genomic profile with variable complexity even if less complex than LMS's profile and it is still unclear how these benign tumors have similar genomic alterations as LMS.

Uterine LM develops through distinct mechanisms with variable prevalence in the different histological subtype of LM (12). The three major genomic alterations involve MED12 mutations, HMGA-2, HMGA-1 aberrations and biallelic FH inactivations as well as COL4A5/A6 deletions (13), (12).The conventional LM type harbors mutations in exon 2 of MED12 subunit in 60% of the cases (from 31 to 92%) (11, 12), followed by HMGA-2 rearrangements (12). LM-BN show a higher percentage of FH inactivations (from 33% (12) to 70% (8)) but less frequently HMAGA-2 inactivation (up to 25%) (14). In the LM-BN group without MED12, FH or HMGA2 alterations, TP53 and RB1 are more frequently altered genes (8). High throughput and integrated data analyses of uterine LMS have shown a huge heterogeneity of

alterations without a driver mutation/translocation (15) (16), the most altered genes being tumor protein P53 (TP53: 6/19; 33%), alpha thalassemia/ mental retardation syndrome X-linked (ATRX; 5/19: 26%), and MED 12 (4/19; 21%) (15).

The diagnosis of conventional LM and LMS is in general straightforward. However, sometimes the diagnosis could be very challenging. A uSMT that cannot be unequivocally diagnosed as benign or malignant should be termed smooth muscle tumor with uncertain malignant potential (STUMP) (1), and only the outcome will confirm its benign or malignant nature.

Few years ago, a study was published on clinical data, follow up and morphologic features of 29 uterine STUMPs. Using Genomic Index (GI) - an estimation of the degree of chromosomal complexity of these tumors by array-CGH - we were able to split this gray category in two subgroups with different risk of recurrence, outcome and distinct genomic profiles (17). Array-CGH analysis is now a useful tool for the diagnosis of difficult uSMTs, complementary to morphological analysis.

In the last 5 years, many analyses based on whole genome approaches have improved our knowledge of USML biology (15, 18, 19). Nevertheless, the routine diagnostic practice lacks complementary diagnostic tools. For uterine LMS, despite very aggressive clinical features (20), it is difficult to predict the outcome, especially when the diagnosis is made at stage I (tumor confined to the uterus).

Moreover, the tumor grading in uterine LMS, one of parameters of a prognostic nomogram (21) is controversial because by definition LMS diagnosed on the basis of Stanford criteria (4) are high grade. Contrary to other soft tissue sarcomas, the tumor grade does not have a prognostic value in uterine LMS (22). Among the 7 parameters of the nomogram, 3 (presence of regional metastasis, distant metastasis and size) are strictly linked to the FIGO stage (21). Hence, there is a need to clarify these prognosis strategies.

The loss of ATRX or DAXX has been associated with a poor survival (23) (24) as well the loss of progesterone expression in stage I LMS (25). In a recent series of 77 uSMTs (19 leiomyomas, 14 STUMP and 44 LMS with clinico-pathological and genomic correlations), GI with a cut-off =10 was shown to be a predictor of recurrence (p=2.267 e-08) and with a cut-off = 35 is a marker for poor overall survival (p=3,495e−02) in uterine LMS.

Endometrial stromal sarcomas (ESS) are classified into two types, low- and high-grade (1). Low-grade ESS is an indolent tumor with a good survival rate even with multiple recurrences (26). High-grade ESS have intermediate prognosis, between low-grade ESS and undifferentiated uterine sarcomas (UUS)(27, 28). Recurrent translocations have been reported in low-grade ESS (JAZF1 being the most frequently rearranged gene) (29, 30) and high-grade (YWHAE, BCOR)(27, 31-33). UUS is a very aggressive sarcoma without a specific line of differentiation and an exclusion diagnosis (26). UUS has a complex genome, a high number of copy variations (34) and a gene expression profile driven by macrophage infiltration (35).

Uterine Tumor Resembling Ovarian Sex Cord Tumor (UTROSCT) is an uncommon uterine neoplasm with morphological overlap with ovarian sex cord tumor (36). The morphology of UTROSCT is variable and the immunophenotype is polyphenotipic as epithelial, smooth muscle and sex cord markers as well hormone receptors could be expressed (37-40). Similar to ovarian sex cord tumors, UTROSCTs express FOXL2 and SF-1 proteins in variable proportion from 40%-53% (41) and 58% (42), respectively. The histogenesis is still unknown and is not related to ESS as UTROSCTs lack JAZF1 often harbored by ESS (43). UTROSCT is regarded as "uncertain" or "low malignant potential" tumor (44). In a recent series with clinico-pathological correlations, up to 24% of the patients recurred or developed distant metastases and 8.8% died of disease (45). No reliable prognostic parameters are available until now.

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INVITED LECTURES

L 23

ABSTRACT FOR KEYNOTE LECTURE

"FROM BETHESDA TO PARIS AT LAST: THE VALUE OF STANDARDIZED TERMINOLOGY IN PATHOLOGY".

Ritu Nayar, MD

In 1988, standardized terminology for reporting cervical cytology (Pap tests) was introduced. The Bethesda System was based on three fundamental principles: terminology must be (1) clinically relevant, (2) reasonably reproducible and flexible, and (3) reflect the most current understanding of cervical neoplasia. This lecture will review the importance of standardized reporting in pathology, the history and contributions of the Bethesda System towards research and management in cervical cancer and its impact on development of additional reporting guidelines for thyroid, urine, salivary gland and pancreaticobiliary in cytopathology and for HPV related squamous lesions in histopathology. The reality of atypia and use of ancillary/molecular testing to refine pathology interpretations, supported by lessons learnt from interobserver reproducibility studies initiated by the Bethesda group will also be discussed.

L 24

TRIPLE NEGATIVE BREAST CANCER: HISTOLOGICAL SUBTYPES.

K. Lambein
Gent

Since the landmark paper by Perou and Sorlie on molecular portraits of breast cancer, the molecular and clinical heterogeneity of breast cancer is well established.

Despite the complexity of the matter, the subtyping of breast cancer in routine practice still relies on the accuracy of hormone receptor and HER2 testing. The lack of these three markers defines triple negative breast cancer.

In general, patients with triple negative breast cancer have worse prognosis compared to hormone receptor positive and/or HER2 positive breast cancer patients. Triple negative breast cancer is known to have more aggressive characteristics and lacks the benefits of targeted therapy. This has lead to intensive research of this type of breast cancer with few therapeutic options.

On the basis of gene expression profiling, several subtypes of triple negative breast cancer have been defined, such as basal like, mesenchymal like, immunomodulatory and luminal androgen receptor subgroups. Some of these molecular subgroups correlate well with histological subtypes of triple negative breast cancer. From a histological point of view, the group of triple negative breast cancer is a very diverse one. Triple negative breast cancer comprises no special type cancers, special type cancers such as those with medullary features and metaplastic carcinomas, as well as several very rare subtypes.

An overview is given of these histological subtypes of triple negative breast cancer, including some recently defined entities. The salivary gland type tumours form a distinct group and will be discussed separately.

If applicable, correlation between histological and molecular subtypes is discussed and possible therapeutic targets. The matter of BRCA mutations and BRCAness will be discussed separately as well as the topic of tumor microenvironment and immunotherapy.

Triple negative breast cancer, previously considered as orphan cancer, has become a hot topic of research and clinical studies, leading to a more personalized therapy.

INVITED LECTURES

L 25

SALIVARY GLAND TYPE TUMOURS OF THE BREAST.

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Introduction:

Salivary gland type breast tumours (SGT-bt) represent a heterogenous group of uncommon breast lesions with diverse biological potential [1]. Morphologically SGT-bt are analogous to tumours that are commonly seen in salivary gland. This similarity is perhaps due to the common ectodermal embryological origin of these organs.

The vast majority of SGT-bt lack estrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor 2 (HER-2) expression, and are therefore classified as triple negative breast carcinomas. Additionally, on immunohistochemistry SGT-bt may express markers that are related to the so called "basal-like" phenotype. However, despite their triple negative status most SGT-bt behave clinically as lesions with low malignant potential.

John Azzopardi was the first author who recognized the morphological similitude between tumors of the breast and salivary glands[2]. Recently it has been proposed that this group of tumors may originate from progenitors cells expressing basal type cytokeratins (CK5/14). These CK5/14 positive progenitor cells may subsequently differentiate into glandular, myoepithelial, mesenchymal or squamous cell lineages [3]. Accordingly, SGT-bt can be morphologically subdivided in three major groups based on their putative tumor-cell differentiation[1]:

1. **Tumors with pure myoepithelial cell differentiation**
2. **Tumors with mixed myoepithelial and epithelial cell differentiation**
3. **Tumors with pure epithelial cell differentiation**

Generally speaking, SGT-bt show a low mutational rate and low level genetic alterations. Notably a subset of SGT-bt is characterized by the presence of specific recurrent homologous translocations. Intriguingly, the same type of translocation may be found in the correspondent lesions of the salivary gland. Unlike the more common types of triple negative breast carcinomas (ie ductal), SGT-bt show less frequently *TP53, PIK3CA, BRCA1* and *RB1* gene mutations[4]. Therefore SGT-bt form a distinct category within the triple negative breast carcinomas, also from the genetic point of view.

Tumors with pure myoepithelial cell differentiation

Tumors composed by a pure (or dominant) population of myoepithelial cells are exceedingly rare. They show often heterogeneous morphology, most likely related to the dual phenotype retained by myoepithelial cells in which at the same time mesenchymal and epithelial phenotypes can be observed. Indeed, they may often show spindled cell morphology, clear-cell, chondromyxoid or squamous differentiation.

Among the benign lesions are described: collagenous spherulosis, myoepithelial hyperplasia and, the exceedingly rare benign breast myoepithelioma[1, 5]. Collagenous spherulosis and myoepithelial hyperplasia are most often in association with other proliferative benign lesion such as epithelial hyperplasia or intraductal papillomas, but on occasion may be associated with in situ carcinomas (DCIS or LCIS). Collagenous spherulosis is a mimick of cribriform ductal carcinoma in situ and is in the differential diagnosis with adenoid cystic carcinoma.

Malignat myoepithelioma or myoepithelial carcinoma is the sole malignant entity of this group. It shows a wide morphological spectrum, widely overlapping also from the immunophenotypic point of view with metaplastic carcinomas. Moreover also clinically metaplastic carcinoma and malignant myoepithelioma show a similar outcome, making their distinction a pure semantic issue. For this reason, in the 4th edition of the WHO book myoepithelial carcinoma is classified under metaplastic carcinoma. Lesions in which is possible to demonstrate the direct origin from the myoepithelial layer of pre-existing ductal structures, can be potentially classified as malignant myoepitheliomas or myoepithelial carcinomas [5-6].

Tumors with mixed myoepithelial and epithelial cell differentiation

Pleomorphic adenoma and adenomyoepithelioma are regarded in the current WHO classification as

benign tumor with mixed epithelial and myoepithelial phenotype.

Pleomorphic adenoma is exceedingly rare in the breast with only a few case reports described in the literature. In contrast, pleomorphic adenoma is the most frequent type of tumor in the salivary glands. Pleomorphic adenoma presents as a well demarcated nodule usually located in the retro areolar region, composed by epithelial cells with mixed phenotype embedded in an abundant chondro-myxoid matrix with sometimes frank osseous or chondroid metaplasia. It shows an indolent clinical behaviour and is mostly cured by complete excision. The most challenging differential diagnosis is with matrix producing metaplastic carcinoma: however, the small size, the association with papillary lesions, the lack of overt atypia, the lack of mitotic activity and necrosis together with peripheral preservation of myoepithelial cells are mostly helpful in confirming the benign behaviour or at most the uncertain malignant potential. Indeed, few reports in the literature have described recurrence after resection and, rarely signs of aggressive behaviour or malignant transformation (e.g. lymphovascular invasion, distant metastasis etc.)[1, 5]. For these reasons recently, it has been proposed to consider pleomorphic adenoma of the breast a lesion with uncertain malignant nature rather than a purely benign lesion[6-7]. No specific genetic alterations are reported.

Adenomyoepithelioma occurs usually in postmenopausal women under the form of centrally located nodular masses without calcifications. On microscopy expansion of the myoepithelial compartment is observed, with multiple layers of myoepithelial cells in close association with glandular spaces. Tubular or papillary patterns are frequently described, but a solid-lobulated pattern or a mixed pattern can be also found. Lesions are most frequently sharply delineated. Myoepithelialcells may show spindled cell morphology or epithelioid morphology with usually clear cytoplasm. The glandular elements are lined by columnar cells with eosinophilic cytoplasm with sometimes apocrine changes, sebaceous changes or squamous metaplasia. Usually the mitotic activity is very scant (< 2 mitoses in 10 HPF). In the presence of signs of aggressive behaviour, such as nuclear atypia, high(er) mitotic activity, perineural invasion or invasion into adjacent breast tissue the diagnosis of atypical adenomyoepithelioma may be rendered. However, clear-cut criteria specifying this category are poorly defined[1, 5-7]. The assessment of the prognosis may be extremely challenging, even in absence of an overt malignant component. Indeed, the occurrence of local relapses or even distant metastasis of otherwise "benign" adenomyoepitheliomas is reported in the literature. However complete resection with clear margin should be curative in most of the cases lacking signs of aggressive behaviour.

Adenomyoepithelioma with carcinoma refers to the malignant transformation of either one or both cellular compartments, and it is characterized by a wide variety of breast carcinomas in combination with the preexisting adenomyoepithelioma. Adenomyoepithelioma with carcinoma shows higher tendency to relapse locally and present a significant metastatic potential, most likely dependent on the grade of differentiation of the malignant component. No specific genetic alterations are described in adenomyoepithelioma with or without carcinoma.

Adenoid cystic carcinoma (AdCC) in the mammary gland represents < 0.1 % of all breast carcinomas. It has a low malignant potential, presenting usually as a well delineated mass in the sub-areolar region of adult female patients. It is composed by a mixture of luminal cells, myoepithelial cells and basaloid cells arranged in cribriform structures. Luminal cells are arranged around true glandular spaces that are often difficult to be recognized. Myoepithelial and basaloid cells are arranged around the so called pseudolumina which are formed by stromal invaginations of various shapes and size. The true glandular spaces contain PAS positive mucins, while the pseudolumina may contain Alcian-blue positive mucins or small spherules or cylinders of basal lamina. Sebaceous changes or squamous metaplasia may be observed in the luminal component. Luminal cells express CK 7 and 8/18, myoepithelial cells and basaloid cells are positive for CK 5, 14, p63, calponine and αSMA on immunohistochemistry [1,4-5]. The expression of myoepithelial markers may be widely variable. Similarly to what observed in the salivary glands, strong KIT expression is consistently observed also in the breast [8]. AdCC is classically described as a triple negative breast tumor, however recent evidence suggests that up to 25% of these tumors may show weak ER expression [1, 9-10]. From the molecular point of view, AdCC is characterized by a recurrent chromosomal translocation involving the genes MYB and NFIB in the majority of the cases[11]; MYB expression by immunohistochemistry may also be observed [12]. The same translocation is found in the salivary counterpart. The description of the panorama of somatic mutations in breast AdCC has revealed a rather low rate of

mutations characterized by frequent mutations in *MYB, BRAF, FBXW7, SMARCA5, SF3B1 and FGFR2*. This is in contrast with what is usually described in ductal-type triple negative breast carcinomas, in which *TP53* and *PIK3CA* mutations are more frequent[12-14]. The mainstay of treatment for conventional AdCC is surgery, given the low-grade malignant potential. Tumor grade according to the criteria adopted for the salivary counterpart, may further stratify AdCC and may identify tumors with higher grade of malignancy. Notably the solid variant of AdCC, which is characterized by a predominant population of monotonous basaloid cells arranged in solid nests, show a higher tendency to local recurrence, lymph node metastasis and distant metastasis [1, 4-5].

Tumors with pure epithelial cell differentiation

Four different types of tumor belong to the group of SGT-bt with pure epithelial cell differentiation: acinic cell carcinoma, secretory breast carcinoma, mucoepidermoid carcinoma and polymorphous carcinoma.

Acinic cell carcinoma is characterized by the presence of zymogen-type cytoplasmatic granules like those described in the acinic cell carcinoma of the parotid gland. To date not more than 50 cases have been described in the literature. Histologically acinic cell carcinoma show a mixture of well differentiated small glands and less well differentiated solid nests with sometimes comedo-type necrosis [1, 4-5]. The distinctive aspect of the cells helps in making the diagnosis: the tumor cells have abundant cytoplasms filled with eosinophilic to amphophilic granules and irregular ovoid nuclei with single nucleoli. The granules may be highlighted by PAS staining after diastase digestion and sometimes may be bright red in colour like in Paneth cells. Clear cells with "hypernephroid" aspect may predominate. The tumor cells are consistently positive for α1-antichymotrypsin, salivary gland amylase, lysozyme, EMA and S100. Acinic cell carcinoma of the breast are triple negative tumors. Based on molecular evidences coupled with elegant morphologic studies, it has been recently postulated that acinic cell carcinoma may belong to the family of ductal triple negative breast carcinomas, representing the low-grade arm of triple negative breast carcinomas of ductal origin. Additionally, by comparison with microgaldular adenosis, a neoplastic proliferation with uncertain malignant potential, it has been also proposed that microglandular adenosis may represent a non-obligate precursor of acinic cell carcinoma. Mutation that are frequently found in acinic cell carcinoma and that are indeed shared by ductal-type triple negative tumor include: *TP53, PIK3CA, MTOR, CTNNB1, BRCA1, ERBB4, INPP4B and FGFR2* [15-16]. Acinic cell carcinoma is an indolent tumor; most patients are alive with no evidence of disease 6-184 months after diagnosis (mean 42 months). Distant metastasis and death has been reported only sporadically[1].

Secretory breast carcinoma also known as juvenile breast carcinoma, was regarded as pediatric breast tumor [17]. The analysis of a large cancer register has revealed that the median age at presentation is 53 years with a male/female ratio of 1/31 and represents < 0.15% of all breast cancers [18]. Secretory carcinoma is a low-grade, translocation associated carcinoma that only recently was found to share similar morphological and molecular features with the mammary analogue secretory carcinoma of the salivary glands [4]. Despite the name, it does not have any relation to pregnancy or lactation. Secretory carcinoma presents most frequently as sharply defined mobile mass in the retro-areolar region in absence of nipple discharge. Under the microscope the tumor presents a well-defined lobulated appearance with focally a more pronounced infiltrative growth pattern. The tumor shows a combination of three main architectural patterns: tubule-alveolar, microcystic and solid. Production of intracellular and extracellular secretion is always present. The tubule-alvaolar pattern shows irregular glandular structure with wide open lumens often filled with intense eosinophilic secretions; microcystic areas merge often with the solid component and mimick thyroid follicles. A papillary growth pattern may also be observed and on occasion may be the dominant feature; rarely extracellular mucin production is also described[19-20]. The tumor cells are polygonal with amphophilic cytoplasm, finely granular or vacuolated. In case of abundant intracytoplasmatic secretions the cells may assume a "bubbly aspect". The nuclear atypia is minimal to moderate, mitoses are rare. Apocrine changes are frequently observed; signet ring cell differentiation is also described. Carcinoma in situ with similar secretory features is associated in 46% of the cases, showing solid or papillary architecture. The bland architectural and cytological appearance may reflect the low aggressive potential of most of the secretory breast carcinomas. Akin to the morphological features, the presence of a recurrent translocation involving *NTV6-NTRK3* genes together with a simplex genomic profile by array-CGH are further argument to support the rather low aggressive profile of this tumor [4]. However, transformation into high grade and clinically more aggressive forms have been reported in the

literature even in the presence of the peculiar *NTV6-NTRK3* translocation[21]. Secretory breast carcinoma is usually strongly positive for S100, shows expression of several basal markers and it might be weakly positive for AR; GCDFP-15 expression is usually absent. Interestingly, ER and PR expression at low level may be occasionally seen in secretory breast carcinomas, HER-2 is mostly negative [21-22]. The efficacy of specific small tyrosine kinase inhibitors against NTRK3 activity is currently being tested in patients carrying secretory carcinomas in various organs [23].

Mucoepidermoid carcinoma is one of the most common type of tumors in the salivary glands. It is exceedingly rare with estimated incidence of 0,3%[1,4-5]. Microscopically, mucoepidermoid carcinoma is characterized by a mixture of different components: basaloid cells, intermediate cells, epidermoid and mucus secreting cells. Tumor cells form solid structures admixed with cystic space and mucine production; the basaloid cels are generally located at the periphery of the solid structures and merge gradually with the intermediate, epidermoid and mucus secreting cells. Mature keratinization is not a feature of mucoepidermoid carcinoma and if present should exclude its diagnosis and favour instead that of metaplastic carcinoma. The cellular zonation is highlighted by immunohistochemistry: the basaloid, intermediate and epidermod cells express p63, EGFR1 and high molecular weight cytokeratines, the mucus secreting glandular elements are positive for CK7 and 8/18. Mucoepidermoid carcinoma is generally negative for ER, PR and HER-2. Tumor grading is important to stratify in prognostic meaningful categories patients carrying mucoepidemoid carcinoma. The *MECT1-MALM2* translocation is considered the genetic hallmark of mucoepidermoid carcinoma of salivary glands[1, 4]. Interestingly, the gene *MALM2* may also be implicated in the pathogenesis of mucoepidermoid carcinoma of the breast because of the presence of one reported case carrying deletion in the *MALM2* locus on chromosome 11q21 [24].

Polymorphous carcinoma is a tumor showing similar histological features to those described in the salivary glands. It is most likely a underrecognized type of breast carcinoma with not more than five cases reported in the literature so far [1, 4-5]. The main differential diagnosis is with invasive lobular carcinoma, or the solid variant of AdCC. The tumor is composed by solid nests of variable size surrounded at the periphery by indian files, alveolar structures, and trabecular or cribriform structures. Unlike AdCC, polymorphous carcinoma is characterized by a single cell-type with round to ovoid nuclei and brisk mitotic activity. Tumor cells are positive for BCL-2, weakly positive for CK7 and maintain E-cadherine expression. Staining for EMA, ER, PR, HER-2, CK14, actin and KIT are consistently negative [25]. The biological behaviour and morphological features are those of a high-grade carcinoma. Interestingly the salivary gland counterpart of polymorphous carcinoma is characterized by recurrent mutations in the *PRKD1* gene [26].

Conclusions

SGT-bt represent a rare and highly heterogeneous group of tumors that in most of the cases belong to the group of triple negative breast carcinomas. Some of them carry very distinctive genetic aberrations and are characterized by a rather low malignant potential, as compared to ductal-type triple negative carcinomas.

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INVITED LECTURES

L 26

TUMOUR-INFILTRATING LYMPHOCYTES AND PDL-1 IN BREAST CANCER.

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Breast cancer (BC) is the most frequently diagnosed malignancy among women, representing about one-third of all new cancer cases and the second leading cause of cancer death after lung cancer. BC development and progression is dependent on a complex system of different factors, including genetic and epigenetic alterations, and on factors from the tumour microenvironment, such as stromal and immune cells. In fact, in recent years, numerous studies have focused on the presence and function of the host immune system and its relationship with tumour progression in a variety of solid tumours, including BC, showing that intratumoural lymphocytic infiltrate is related to patient prognosis and can serve as guide for therapy. Recently, large cohort studies have shown an association between the presence of tumour-infiltrating lymphocytes (TILs) with improved prognosis and better response to neoadjuvant chemotherapy. In triple-negative (TN) BC, the presence of stromal TILs is associated with better outcome after adjuvant anthracycline-based chemotherapy.Similarly, in human epidermal growth factor receptor 2 (HER2)-positive BC, the number of TILs in tumour tissue associates with a better response to trastuzumab treatment. It was also shown that tumour cells express antigens that should be recognised by patient's immune system, although most of the time the immunological response is unable to eliminate the cancer cells. Currently, many efforts have been made to identify molecular mechanisms that enable tumour cells to escape from the host immune system. An example of tumour escape from immunosurveillance is the expression of PDL1 (programmed cell death-ligand 1) by neoplastic cells, which is a cell surface glycoprotein that conveys an inhibitory signal to T lymphocytes, through the interaction with its receptor PD1 (programmed cell death protein 1). This specific binding leads to a decrease in cytokine production and an increase of T lymphocyte apoptosis, which protect tumour cells from elimination. Accordingly, the inhibition of this inhibitory signal by specific monoclonal antibodies, against either PDL1 or PD1, has been shown to promote tumour cell death induced by the host immune system in many cancer models. Based on these data, the expression of PDL1 is already being evaluated in several solid tumours, including breast cancer. A recent study from our group confirmed that increased stromal TILs are present in a minority of invasive breast cancer (IBC) cases and that are already present in in situ stages. Although PDL1 expression has

been reported in the literature from 20% to nearly 60% of the cases, we only observed in a small proportion of IBCs (less than 10%) and, for the first time, even in DCIS cells.In IBC, increased stromal TILs and PDL1 expression were associated with each other and with G3 and TNBC subtype, with statistical significance in both cohorts, which also validates previous reports. The association of increased stromal TILs and PDL1 expression with the evaluated basal cell markers also reinforces the relationship with the TNBC molecular subtype. Different values for the expression of PDL1 can be related with the antibody used and the methodology of quantification. In our case we use the clone SP142 and positivity was defined as membranous and cytoplasmic staining in more than 1% of tumour cells and or stromal TILs. Other studies also showed that besides the NST carcinomas, PDL-1 positivity is more frequently in medullary, metaplastic and apocrine carcinomas and a good correlation between positivity in primary and metastatic tumour. In conclusion, TNBC and HER2 positive carcinomas contain more TIL and are more likely to be PDL1 positive than luminal (ER+) carcinomas, and thus are the most attractive candidates for immunotherapy. In fact, clinical trials using anti PD1 and PDL1 have showing good results in TNBC. However, there are still some challenges to be overcome in breast cancer: is assessing TILs on HE sections alone enough? Is TILs scoring ready for primetime use? How de we standardize PDL1 assessment? What is the role of multiplex assays (CD8, PDL1, FoxP3?) What is the impact of the cancer mutation load in breast cancer?

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L 28

"THE 2014 BETHESDA SYSTEM FOR REPORTING CERVICAL CYTOLOGY: PATTERNS AND PITFALLS IN SQUAMOUS AND GLANDULAR LESIONS".

Ritu Nayar, MD

This lecture will review the reasoning behind the 2014 update of the Bethesda System for Reporting Cervical Cytology. The focus of the lecture will be on the morphology of high grade squamous and glandular lesions. The basic criteria for interpretation are key for cytotechnologists and pathologists alike. Classic and less common appearances of HSIL/AGC/AIS will be presented. Lookalikes and mimics of high grade lesions will be shown and clues to avoid these pitfalls will be discussed. Images from the Bethesda interobserver reproducibility studies will be used to discuss areas of difficulty and less concordance among observers. Resources on the Bethesda websites will be shown.

INVITED LECTURES

L 30

"NEXT GENERATION SEQUENCING ON CYTOLOGICAL SAMPLES".

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Molecular cytopathology, a rapidly evolving field of modern cytopathology, features an increasing number, variety, breadth and depth of tests, which underlines the effective interplay between genomics and cytology.[1] Challenging cases classified as atypical or as of undetermined significance may be further stratified into high- and low-risk groups by the demonstration of specific oncogenic mutations.[2] Moreover, by the development of personalised/ precision medicine, cancer gene testing on cytological samples from patients with surgically unresectable, high-stage locally advanced, recurrent, or metastatic malignancies is crucial.[3] Although, fine-needle aspiration (FNA) biopsy, a rapid, efficient, and minimally invasive technique, and core needle biopsy (CNB) represent complementary methods to sample superficial and deep-seated lesions, the use of FNA for gene testing is advantageous over CNB in several respects. Despite a wide range of cytopreparations, fixation, and staining techniques, FNA have higher tumour fraction, ensure a wider sampling of the targeted lesion, offer a better quality DNA and an effective triage for ancillary studies when coupled with rapid onsite evaluation.[2] More recently, cytological specimens have also been validated for next generation sequencing (NGS) to simultaneously screen different types of mutations in multiple genes and in multiple patient samples using small amounts of input material.[4]

The cytopathologist is responsible of those multiple actions cumulatively referred to as pre-analytical processing He has to review cytopathology reports and archived materials to select best quality smears or representative cell block sections to determine the cellularity and purity of the tumor sample being submitted for biomarker testing, having the responsibility to cancel the request for molecular assay whenever the cellularity is below the analytical sensitivity of the molecular assay. Similar to surgical pathologists, there is a wide interobserver variation also among cytopathologists in estimating tumor fraction and even in the same institution cancellation rates vary widely among cytopathologists.[5] Care should be taken to identify viable tumor areas in which the tumor ratio is optimal and the percentage inflammatory cells and of potentially amplification inhibitors (such as mucin, melanin and tumor cell necrosis) is minimal.[2] Since various mutational assays have different analytic sensitivities, the cytopathologist (or the technician) should enrich for tumour content to a level that is acceptable for the assay being used. Once the results of the genotyping analysis are received, the cytopathologist needs knowledge of the molecular diagnosis and of available treatment strategies; on occasion, the cytopathologist may also compare the mutation signals with the extent of tumour cells in the tested specimen, carefully evaluating the quality processes employed to ensure confidence in the results, taking care to integrate the molecular data in its original diagnostic report.

As a general rule, the test request should be made appropriately to ensure that every patient who needs a test is offered one in a timely manner, while avoiding unnecessary procedures. The test is usually requested by the oncologists and less frequently by other specialists, including surgeons and interventional radiologists. Ideally, rather than by a single specialist, test request should be made multidisciplinary (tumour board).[6] Also in light of the increased awareness among patients and their families of the novel technological and therapeutic opportunities, the tumour board should ensure that the needs of a precise cytological diagnosis and of multiple predictive assays would simultaneously be met.[6] Thus, the effective communication between the laboratory, the oncologists, and the cytopathologist is crucial to plan effective sampling strategies to ensure that adequate tissue amount is obtained.[7] As a matter of the fact, the cytopathologist may not know whether the patient is a candidate for surgery or for targeted therapy. Thus, the cytological sample is not the optimal testing approach when a larger resection specimen is subsequently available for analysis.[7] Similarly, for diagnoses made on a metastatic or recurrent lesion, the cytopathologist should be informed whether any prior specimen of the same patient has already been tested. Previous chemotherapy regimens can change gene expression and mutation

status and should be documented on the request form. In some cases, patients with poor performance status may still be considered candidates for testing, as clinical response without significant side effects may follow the detection of a targetable genomic alteration.[7] Rather than on oncologist's demand, the automatic (reflex) testing by cytopathologists, based on diagnosis and tissue availability, can be more efficient. Reflex testing avoids the costs in time and money of specimen retrieval from pathology archives and the treatment delay for patients who are found to harbour a targetable molecular alteration.[8] However, molecular testing is expensive and as molecular biomarkers are evolving rapidly over time, new targets may be identified in the interval between diagnosis and recurrence.

As technology is advancing at rapid pace, a range of novel techniques is emerging. In particular NGS and fully automated platforms may necessitate specific sample requirements and dedication from cytopathologist to develop special cytopreparation protocols. In particular, establishing the minimum number of cells needed to allow a next generation sequencing approach from cytology sample is a crucial point. The studies that applied NGS to cytological material had usually a retrospective design and only samples that featured at least 20% of neoplastic cells were selected, which may not fully reflects current practice. In any case, sample requirement depends on target capture, gene panel and platform types. Illumina NGS required 15.000 cells when following hybridatation capture or 5000 cells when preceded by PCR based capture, while Ion Torrent NGS needed between 100 and 1000 cells. As far as DNA input is concerned, Illumina NGS required from 50 to 170 ng, following hybriditation capture or 30 ng downstream of multiplex PCR. Conversely, Ion Torrent sequencing of PCR products, only needs 10 ng of DNA and precisely 12 µl of diluted DNA at a concentration of 0.8 ng/µL.. Even more recently, it was shown that lowering the input DNA concentration below the manufacturer's recommended threshold of 10 ng (>0.8 ng/µL) is feasible leading to a marked increase in the NGS success rate from 58.6% to 89.8%. [9] More relevant than DNA input, is the percentage of neoplastic cells; in a low cancer cell background, the preferential amplification of a small number of DNA molecules may be representative only of the benign component, leading to a false negative result. As a matter of the fact, most NGS assays have a lower limit of mutation detection of 10%, which requires at least 20% of neoplastic cells However, a more recent NGS approach, based on the use of narrower gene panel focused on a limited number of targets enables the detection of low abundant mutations with a specificity of 100%.[10]

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APPLICATION OF IMMUNOCYTOCHEMISTRY IN CYTOLOGY SPECIMENS.

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The use of immunohistochemistry (IHC) techniques in cytology has expanded rapidly over the decades. Akin to the situation in histopathology it has led to an increased diagnostic accuracy. In addition, tumor cell characteristics that determine sensitivity to targeted therapies can also be defined on cytological material. The main challenges for the application of IHC on cytology are to select the proper test for a limited sample quantity, to avoid jumping blindly applying the protocols that have been set for histology to cytology and to use appropriate controls for cytological material. Concerning IHC, there are studies in the literature showing some lack of standardization in the application of this technique to cytological material. There are many potential causes that can explained the variability of results, such as the use of improper controls, use of non-customized reagents concentrations, different methodologies of fixation and preparation of the material, among others. It is important emphasize that cytopathologists should avoid apply the same conditions used on histological material on the cytological material. Unfortunately, this quick jump from a histological section to a cytological specimen is frequently done without consideration of the major differences in the specimen preparation that can affect the final interpretation of the reaction with potential clinical consequences for the patient. Comparison with a standard procedure as in paired samples with histological biopsies is a good example of validation. In some clinical situations cytological samples are the only material available for testing. Therefore, validation is essential to obtain accurate results.

Cytology laboratories process different types of material such as fine needle aspirates (FNAs), cell suspensions and various types of exfoliative collections. Proper specimen processing is of utmost importance for any ancillary technique. The most commonly used preparations are direct smears, cytospin centrifugations, cell blocks and liquid-based cytology (LBC) preparations. Direct smears are prepared from FNA material, brushings or sediment fresh effusions. After being air-dried they should be fixed in formalin followed by alcohol and perform well in immunostaining for nuclear antigens such as Ki-67, TTF-1, ER; they are, however, less suitable for staining of membrane or cytoplasmic antigens because of high background staining due to cell damage caused by the smearing. The cytomorphology of direct smears is excellent but the number of slides, in particular from FNA biopsies, is usually limited and will prevent the use of an extensive marker panel. Although the cost of preparation of direct smears is low, the amount of antibodies needed to cover the entire slide is relatively high. Cytospin preparations are prepared from cell suspensions in non-fixative solutions such as PBS, RPMI, etc., of FNA material or effusions. Cytospin material offers an excellent source of staining for most antibodies although various fixatives have to be used. The technique is less suitable for specimens with a rich admixture of blood or mucous. In most cases a large number of cytospin slides can be prepared, which allows the use of panels with many antibodies. At present cytospin material is one of the choices for evaluation of lymphoid lesions. However, in our daily practice flow cytometry from cell suspensions had been the preferential option in the work-up of lymphoproliferative lesions.. Air-dried cytospin slides can be stored for many years at -70C without loss of antigenicity and excellent DNA preservation. LBC preparation systems are today available in most cytology laboratories. They can be used for almost all types of cytological specimens. LBC preparations can be stained with various antibodies to nuclear, cytoplasmic and membrane-bound antigens in a reproducible way. Unfortunately the material is not optimal for evaluation of lymphoid neoplasms. Several slides can be produced from LBC material, which allows a complete immunological work-up in most cases. The cytomorphology of LBC material is different to that of direct smears, which can initially be a restraining factor. The cost for LBC preparations is higher than for other types of material. LBC slides, post-fixed in 95%

ethanol, can be stored for months at -70ºC without change in immunoreactivity. In a multinational study, cytospins and monolayer preparations were superior to direct smears for the evaluation of estrogen (ER) and progesterone receptor (PR) on breast carcinomas samples obtained by FNA. Cellblocks can be prepared from all types of cytological specimens, except preparations with low cellularity such as cerebrospinal fluids. There are several techniques to produce cellblocks, such as cytocentrifugation, either with direct formalin fixation or with fixation after addition of plasma-tromboplastin. In addition, there are commercially available systems, which offer a standardized technique with a high reproducibility. Cellblocks perform in a highly reproducible way when stained with most antibodies, except for some used in the work-up of lymphoid lesions. One distinct advantage of cellblocks is that many slides can be prepared for extensive panels of immunostains. In addition, the quality control of cellblock staining is identical to that of histopathology. The morphology of cellblocks is identical to that seen in histological specimens and therefore familiar to most pathologists. The technique, which is available in most laboratories, is relatively time-consuming and the cost is comparable to that of the cytospin technique.

Fixation is needed for all types of IHC and the choice of procedure is determined by the type of routine cytological staining preferred, and by the various antibodies and their epitopes used. There are a number of fixation procedures used, which are based on various fixatives such as ethanol, methanol, acetone and formalin. The choice of fixative is of utmost importance for an optimal performance of immunocytology. In fact, IHC techniques are more sensitive to the type of fixation than molecular techniques. The variability in fixatives is one the major factor preventing standardization of some procedures using IHC. Nuclear antigens such as ER, PR, AR, CDX2, TTF-1, p63, WT-1, PAX2, PAX8 and MIB-1 perform best after fixation of air-dried specimens in buffered 4–10% formalin followed by methanol-acetone. Microwave retrieval can be used instead of methanol-acetone treatment. Formalin fixation also appears to be reliable for detection at the HER-2 with immunocytochemistry as well as ISH analysis of the HER-2 gene. Commercially available LBC fixatives have been used with good results for detection of nuclear epitopes. Alcohol-fixed, air-dried or a combination of these fixatives have been reported to give less reproducible staining with antibodies to nuclear epitopes. Methods of fixation and antigen retrieval were the key points in obtaining good results in a comparative multinational study of ER and PR detection in breast cancer FNAs. Membrane and cytoplasmic antigens seem in most instances to have less stringent requirements for type of fixative. Air-dried smears fixed in formalin follow by ethanol generally give optimal staining results. Some authors recommended that the choice of fixative should be determined by the particular antigen studied . Thus, for hematopoietic markers acetone fixation was suggested while for non-hematopoietic markers a 1:1 mixture of methanol-absolute alcohol performed best. For cellblocks, buffered formalin is suggested as the optimal fixative by most authors. Fixative and reagent concentrations should be adapted for cytology. Certain markers, such as gross cystic disease fluid protein 15 (GCDFP-15), S100 protein, or Hep Par 1, are leached from alcohol fixatives and can render false-negative results. Our recent personal experience has demonstrated that important predictive markers, such as antibodies anti-ALK do not perform well in LBC fixatives. Reagent concentrations should be customized for cytological specimens; otherwise, you can have false-positive results because of the excess of antibodies.

The basis of a good and valid technique is a correct choice of positive and negative controls verifying the sensitivity and specificity of the technique. A meta-analysis study demonstrated that in more than 50% of published papers about IHC applied on cytological material, controls were not even mentioned.

IHC methods have been refined and high quality reagents and automation is more widely available, so more and more the technical problems have been reduced in the clinical practice. Automated IHC offers the opportunity to reach high levels of quality, reproducibility and consistency while reducing labour and reagent costs. Therefore, the fact that the majority of laboratories are using automated IHC on cytological samples is a decisive step towards standardization.

There are many possible applications of the use of IHC in different organs and cytological material. In the lecture, we will focus in the most frequent applications and in the organs that the author has more experience. In this text, just a brief summary in some organs:

INVITED LECTURES

1. Lung cytology: Lung cancer is the solid tumor for which diagnosis and therapeutic decisions rely more frequently on morphologic evaluation performed in small biopsies or cytological material. Often, cytology is the only material available for diagnosis in lung cancer; therefore, a panel of well-defined markers must to be used to refine a diagnosis of non-small cell lung cancer (NSCLC) to adenocarcinoma (ADC) or squamous cell carcinoma (SqCC). Therefore, is useful to validate a minimalist IHC panel paying particular attention not to exhaust the diagnostic material jeopardizing an eventual molecular study. There is evidence that a two-hit minimalist approach on cytological material based on p40 and TTF1 are very helpful in the distinction between ADC and SqCC. Recent data with commercially available ALK and ROS-1 antibodies suggests that IHC is a reliable and cost-effective means of preselecting patients for FISH analysis.

2. Serous effusions: The sensitivity and specificity of conventional cytology in the diagnosis of malignancy in effusions are around 57.3% and 89.0% respectively. The reactive mesothelial cells can have architectural and cytological aspects simulating mesothelioma and even adenocarcinoma cells. There are many techniques used for this differential diagnosis such as histochemical staining, immunocytochemistry and electron microscope. Briefly, the reactive mesothelial cells are in general positive for desmin and negative or with a focal and weak positivity for EMA, in contrast with mesothelioma cells that are positive for EMA and negative for desmin. P53 may also be helpful in this differential diagnosis and has been shown to be expressed at much higher levels in mesothelioma. The distinction between mesothelioma and adenocarcinoma cells is based in a panel of markers. Calretinin, D2-40 and WT-1 are the most common used markers for mesothelioma while Ber-EP4, MOC31 and CEA are positive in adenocarcinomas. PAX-8 is another useful marker because is negative in mesothelioma and positive in a high percentage of serous carcinomas [34]. CK 5 is positive in mesothelioma but also in SqCC, in these cases P40 may be valuable because is positive in SqCC and negative in mesotheliomas.

3. Lymph node cytology: In our institution, FNAC is used as a first-line approach to evaluate lymphadenopathies. FNAC yields a high rate of conclusive cytological diagnoses in the assessment of Hodgkin disease or large cell non-Hodgkin lymphomas. The combined use of morphology, flow cytometry (FCM) for immunophenotyping and genetic analysis has increased the accuracy of diagnosis and correct categorization of lymphomas on FNAC samples. In nowadays the use of IHC in aspirates of lymphomas is very limited. In metastatic disease, IHC is the technique more used. The correct identification of site of origin for a metastatic lesion is of utmost importance to allow correct therapeutic measures to be taken. The co-expression patterns of cytokeratin 7 and 20 have been shown to be of great value in suggesting the origin of metastatic adenocarcinomas [4]. In summary, CK7+, CK20+: Pancreatic carcinoma, transitional cell carcinoma; CK7+, CK20 -: Breast carcinoma, endometrial carcinoma, small and non-small cell lung carcinoma; CK7-, CK20+: Colorectal carcinoma, Merkel cell carcinoma; CK7-, CK20: Hepatocellular carcinoma, prostate carcinoma, renal cell carcinoma. There are several subtypes of adenocarcinoma that show the same expression profile but the number of alternatives will be markedly reduced when tumors are categorized with respect to CK7 and CK20 expression. The outcome of this will guide the choice of more site-specific antibodies, such as those against TTF-1 (lung, thyroid), CDX-2 (GI tract), hormone receptors and GATA-3 (breast), PSA (prostate) and calcitonin (medullary thyroid carcinoma).

4. Metastatic cancer cytology (including breast): The tumour heterogeneity in primary and metastatic tumours suggest that to guide therapy in metastatic cancer it will be important to study the characteristics of metastatic cells. Since surgical biopsy of metastasis might be associated with negative outcomes, FNA can be a safe, trustable and cheaper alternative to obtain cells from metastatic sites to study cell characteristics. An important field to assess biomarkers is metastatic breast cancer (MBC). MBC is usually diagnosed by a combination of clinical and imaging findings. Once diagnosed, the choice of systemic therapy is based on the ER, PR and HER2 status from the patient's primary tumor. Biopsy of suspected metastatic lesions is rarely done. However, tumor characteristics can change and discrepancies between primary and metastasis are described with variations until 30% for the hormonal receptors and 5 to 10% in HER2.

The use of IHC in cytological material of urine or FNA from thyroid, soft tissue and gastrointestinal tract is extremely limited.

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Sentinel node biopsy in patients with primary cutaneous melanoma of any thickness: A cost-effectiveness analysis

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ABSTRACT

Objective: To assess the cost-effectiveness of the sentinel node biopsy with lymphadenectomy for nodal metastases (SNB) in patients with primary cutaneous melanoma (CM) of different Breslow thickness (intermediate, thick, thin).
Methods: Decision tree models were constructed to compare two different strategies of management of patients with CM, wide excision of the primary lesion and SNB and wide excision only (WE). Tree models were created for every Breslow thickness over 1-, 5- and 10-year time horizons. Mean and total direct healthcare costs, life years saved (LYSs), quality–adjusted life years (QALYs), cost effectiveness ratio (CER), and incremental cost effectiveness ratio (ICER) were estimated. Every model was considered as a base case, and its results tested with sensitivity analyses.
Results: Base case analyses showed that the best results were obtained for intermediate CM over 10-year time horizon. In this case, ICER for SNB was 130,508€/QALY, well over the threshold of acceptance (30,000€/QALY). In patients with intermediate CM over 1 and 5 years, and for those with thick and thin CM at any time horizon, negative ICER values were estimated since SNB was proved to be more expensive and less effective than WE. Sensitivity analyses confirmed the robustness of our results.
Conclusions: SNB caused no improvement in health outcomes in terms of LYSs and QALYs in patients with thick and thin CM, and only a slight benefit in those with intermediate CM. WE was more cost-effective compared with SNB for any CM thickness over any time horizon up to 10 years.

1. Introduction

Cutaneous melanoma (CM) is a significant health problem in the United States, with approximately 73,870 incident cases expected in 2015 (constituting 4.5% of new cancer diagnoses). Rates for new melanoma of the skin cases have been rising on average 1.4% each year over the last 10 years [1]. In Europe, CM is the ninth most common cancer, with more than 100,000 new cases diagnosed in 2012 (3% of total) [2]. This rapid increase of melanoma incidence constitutes an important economic burden.

Sentinel node biopsy (SNB) is a surgical procedure that consists of the identification and removal of the lymph node(s) that directly receive the drainage from a primary lesion [3]. Despite the widespread use of SNB from its introduction in many countries, no consensus exists on its indications [4,5]. Moreover, the results of the only randomized controlled trial conducted for the evaluation of SNB benefit in CM, the Multicenter Selective Lymphadenectomy Trial 1 (MSLT-1), showed no difference in 10-year melanoma–-specific survival between the two groups of patients compared (wide excision of the primary lesion and SNB with lymphadenectomy for nodal metastases and wide excision only followed by observation) in intermediate and thick CM. However, sentinel node

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(SN) status is associated with differences in progression-free survival for patients with CM [6–8]. SN status is included in the American Joint Committee on Cancer (AJCC) melanoma staging system from 2003 [9].

When appraising a medical innovation, policy analysts and decision makers consider a number of implications, including clinical, economic, and ethical. An estimate of the incremental cost per quality adjusted survival, i.e., cost-effectiveness, is a commonly used composite measure intended to summarize these implications and provide comparisons across patients, diseases, and technologies. They also provide insight through systematic sensitivity analyses on the factors that are likely to most influence the decision. Two studies to examine the cost-effectiveness of SNB in patients with primary CM have been published. One of them [10], performed for thin melanomas, questioned its appropriateness (values for a life saved ranging between 518,000 and 770,000€) and, the other one [11] focused on CM $\geq$ 1 mm, showed an improvement in health outcomes for SNB compared to observation with a slight cost increase (1860€/QALY).

Sentinel node biopsy with lymphadenectomy for nodal metastases in cutaneous melanoma patients (besides characteristics of the primary tumour) is a commonly used staging and treating technique. Despite this, its cost-effectiveness has not been studied using long-term clinical data. In this study, we sought to evaluate the cost-effectiveness of SNB in patients with primary CM of any Breslow thickness, over 1, 5 and 10-year time horizons, from the health care system perspective.

2. Methods

Decision tree models use hypothetical patients that move from one health state to another (transition probabilities) in a simulated natural evolution of a disease, predicting patient numbers in those health states [11]. This type of model was performed for patients with different Breslow thickness CM: thin ($\leq$1 mm), intermediate (1–4 mm), and thick (>4 mm), considering each one as a base case. This segmentation in three thickness classes was done since it is considered to be an important prognostic factor, being worse as it increases [6]. The decision trees were used to compare two strategies of management: (i) wide excision of the primary lesion followed by SNB and lymphadenectomy for nodal metastases (SNB strategy), and (ii) wide excision of the primary melanoma followed by observation (WE strategy). Two different initial health state patients were observed, SN positive and SN negative in the SNB strategy. If SN was positive, complete lymph node dissection (CLND) followed the SNB, and mere observation if it was negative. The final variables, evaluated depending on the health state, disease free, nodal relapse, distant relapse and death, were estimated as life years saved (LYSs) and quality–adjusted life years (QALYs), over 1, 5 and 10-year time horizons (Fig. 1). Institutional board approval was not required since it is a cost-effectiveness analysis and no clinical data have been used.

2.1. Input data

A hypothetical population of 10,000 patients over 18 years old with primary CM was included in every strategy and for every thickness. After that, they were distributed in the different predefined health states, depending on their transition probabilities. Nodal and distance relapse probabilities, SN positivity, and mortality rates for intermediate and thick CM, were obtained for each strategy from the best available evidence, i.e.: the MSLT-1 trial [8,12]. For thin CM, no trials have been published, so global thin CM data were used for WE strategy [13,14] and for SNB strategy, the best evidence found was included [15]. Ten year rates were preferable and were converted in 1-year probabilities following the 1-exp(-rt) formula, assuming the progression probability every year as constant. Disease free survival rate was obtained as 1 minus the rest of the parameters in every strategy. Death was considered as an absorbing state. The model assumed that after a nodal relapse a complete CLND was performed (Table 1).

Locoregional and in-transit relapses were not considered for the model since MSLT-1 results showed no differences between both strategies in 5 years [12]. No differentiation between early and delayed CLND was took into account. Adverse events of SNB and CLND in the operated lymph node basin were considered as intermediate results, and were obtained from complication rates in the MSLT-1 trial. Excision morbidity was not included for being similar in both strategies [16].

Utilities for each stage, that measure both life gained and its quality, with values between 0, death state, and 1, full health state, were extracted from a melanoma population study [17] (Table 1). AJCC staging was considered to fix utilities, not including substaging. Morbidity was considered as a disabling weight, having a negative value [18].

Costs were extracted from Spanish government publications [19–21] and were adjusted to 2015 euros with an interest annual rate of 3%. A 0% discount rate was considered for the case base analysis. Direct healthcare costs were included. According to the National Comprehensive Cancer Network (NCCN) recommendations [22], costs of the different health stages for a group of patients were estimated adding diagnosis, treatment and follow-up costs (Supplementary Table 1).

Total costs of health stages and strategies were estimated (Table 2). Death cost was considered as 0€. SNB surgery costs were weighted depending on the node basin complication rate, 10.1% if no CLND was performed and 37.4% if the SN was positive or if nodal relapse appeared [16]. The adjuvant therapies included were Interferon alpha (INF) in the nodal relapse and anti-CTLA-4 immunotherapy for stage IV, the only drug in monotherapy in preferred regimen with category 1 recommendation [22]. Radiotherapy, surgery and radiosurgery are applied in some patients but were not included because they are not that standardized.

2.2. Model outcomes

LYSs were simulated as the number of patients who would be in every health state. LYSs of death group were estimated because it corresponded with the number of patients that would be in this stage. QALYs were obtained by multiplying the utility by the time spent in every stage. The disutility of complications was subtracted from the total.

For SNB and WE strategies, the mean and the total cost per patient and the effectiveness ratio in €/LYS and €/QALY were estimated for the three CM thicknesses, and the incremental cost-effectiveness ratio (ICER) was also obtained, comparing SNB *versus* WE.

These results allowed performing cost-effectiveness planes, where the WE strategy occupied the origin of the graph, the incremental costs were in the y-axis and the incremental effectiveness, i.e.: the benefits of the SNB, in the x-axis. In this kind of representation, when a new intervention is both clinically superior and cost saving, it is referred as an economically dominant strategy, and falls within the quadrant II. The opposite situation is a dominated strategy (quadrant IV) and, when a strategy falls within quadrant I, more costly and more effective, a threshold to acceptance should be considered.

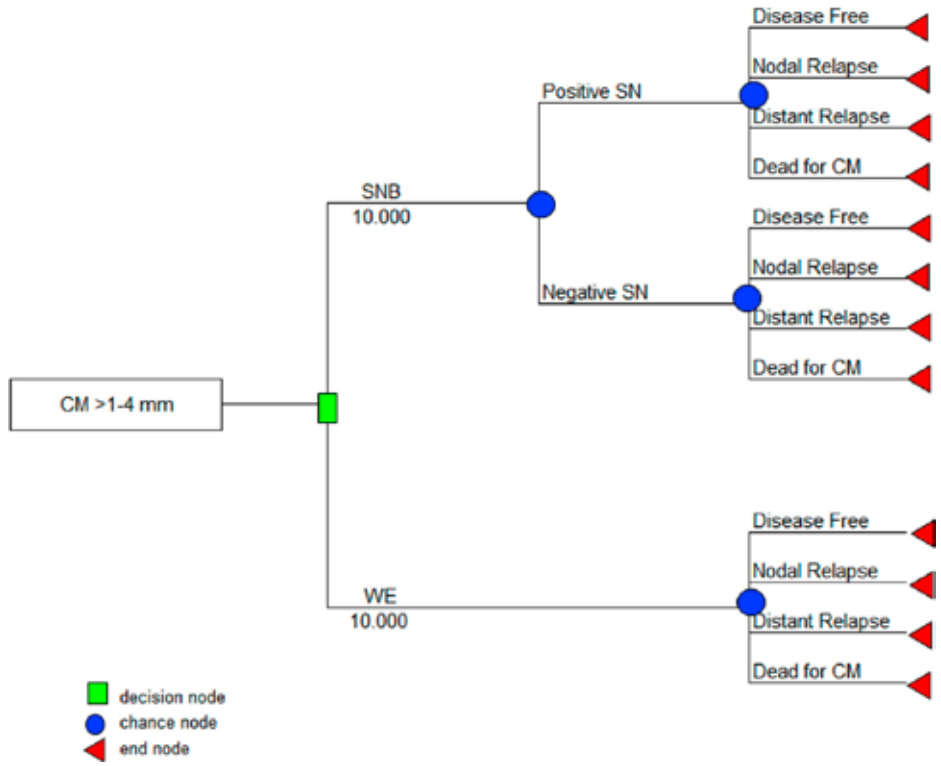

Fig. 1. Simplified tree decision model of intermediate cutaneous melanoma (CM) for sentinel node biopsy (SNB) and wide excision (WE) strategies. Similar models used for thick and thin cutaneous melanoma over 1-, 5- and 10- year time horizons.

Table 1
Clinical probabilities and utilities of cutaneous melanoma.

Clinical probabilities	Intermediate CM[a]	Thick CM[a]	Thin CM[a]
SN positivity	0.159	0.329	0.052
Disease Free Positive SN[b]	0.9271	0.8954	0.9510
Nodal Relapse Positive SN[c]	0.005	0.0092	0.0000
Distant Relapse Positive SN[c]	0.0278	0.0301	0.0247
Mortality Positive SN	0.0401	0.0653	0.0243
Disease Free Negative SN[b]	0.9691	0.9265	0.9266
Nodal Relapse Negative SN[c]	0.0046	0.0099	0.0568
Distant Relapse Negative SN[c]	0.0126	0.0272	0.0089
Mortality Negative SN	0.0137	0.0364	0.0077
Disease Free WE[b]	0.9511	0.9018	0.9847
Nodal Relapse WE[c]	0.0158	0.0410	0.0034
Distant Relapse WE[c]	0.0118	0.0175	0.0036
Mortality WE	0.0213	0.0397	0.0083

Stage[d]. Health state	Utilities		
	Mean	Low limit	High limit
I. Disease Free (<1 mm)	0.926	0.807	1
II. Disease Free and Negative SN (>1 mm)	0.915	0.788	1
III. Positive SN and Nodal Relapse	0.72	0.438	1
IV. Distant Relapse	0.58	0.24	0.92
Loss after CLND with Complications	−0.03		

SN: Sentinel Node; WE: Wide Excision; CLND: Complete Lymph Node Dissection; CM: Cutaneous Melanoma.
Bibliographical sources: clinical probabilities for thick and intermediate CM [8], clinical probabilities for thin CM [15] except for Nodal and Distant Relapse WE [14] and mortality WE [13]. Utility values [17], except for Loss after CLND with Complications [18].
[a] One year probabilities from 5 or 10 years rates using 1-exp(-rt) transformation.
[b] Probabilities obtained as 1-sum of the rest of probabilities.
[c] Probabilities obtained from the first site of nodal and distant relapse MSLT-1, except thin CM. Relapse data from the worst diagnostic.
[d] No subgroups considered.

2.3. Sensitivity analysis

Last, to test the robustness of the results, a one-way sensitivity analysis was performed, modifying the value of the variables in every branch of the tree, SN positivity and mortality rates, utilities, 3% discount rate, and PET-CT in the follow-up. Utilities were adjusted in their high and low level range values at the same time, whereas the rest of variables were modified one at a time, keeping the rest of them constant. A two-way analysis was then performed using the variables that, at the same time, improved the ICER results the most.

3. Results

3.1. Base case analysis

3.1.1. Intermediate CM

Mean cost per patient was 25,822€ following SNB strategy and 22,683€ for a WE patient over 10 years (Table 3). LYSs and QALYs were 0.84 and 0.7 respectively for SNB patients and, 0.81 and 0.67 QALYs for WE patients. QALYs difference between both strategies was 0.024, a week of life with an increase in costs of 3140€ per patient. Cost-effectiveness results showed that ratios for SNB strategy were 30,812€/LYS and 37,125€/QALY, being 28,143€/LYS and 33,780€/QALY for WE strategy, for the same time horizon.

Differences in LYSs and QALYs were negative for SNB strategy the first year, and also were ICER values. Over 5 years, the ICER values were 316,730€/LYS and −1,287,699€/QALY (Supplementary Table 2), and over 10 years, 97,849€/LYS and 130,508€/QALY (Table 3). Negative ICER values were not considered due to their questionable utility, focusing the attention in net benefits [23,24].

Table 2
Main costs in cutaneous melanoma, 2015 prices in euros.

Health state/clinical management	Components	Costs (€)
Excision	First and results visit; Blood tests Day surgery (excision-biopsy); Pathology	2591
SNB, negative SN	Pre-anaesthesia visit and study Lymphatic mapping Surgery (reexcision & SNB)[a] Pathology; Results visit	4774
SNB, positive SN	Pre-anaesthesia visit and study Lymphatic mapping Surgery (reexcision & SNB& CLND)[b] Pathology; Staging study (CT, blood test) Results visit	7186
Nodal relapse	Pre-anaesthesia visit and study Lymphatic mapping; Surgery (CLND)[b] Pathology; Staging study (CT, blood test) Results and Oncology visits INF alpha (5 days × 4 weeks) Outpatient medical oncology	20,839
Distant relapse	Oncology visits; Restaging studies (CT & MRI) Inmunotherapy (Ipilimumab 4 cycles) Outpatient medical oncology	77,399
1 year follow-up. Stage I		313
1 year follow-up. Stage II-IV		2533
2 and following years follow-up. Stage I		157
2 year follow-up. Stage II-IV		1535
Following years follow-up. Stage II-IV		1379
Follow-up from 5 to 10 years		157
Death state		0

SNB: Sentinel Node Biopsy; SN: Sentinel Node; WE: Wide Excision; CLND: Complete Lymph Node Dissection; CM: Cutaneous Melanoma.
[a] 10.1% complication rate.
[b] 37.4% complication rate.

Table 3
Final costs and effectiveness per patient, and rates of SNB and WE strategies in cutaneous melanoma over 10-year time horizon.

Breslow thickness	Strategy	LYS	QALY	Costs (€)	€/QALY	ICER (€/QALY)
Intermediate						130,508
	SNB	0.84	0.70	25,823	37,125	
	WE	0.81	0.67	22,683	33,780	
Thick						−692,074
	SNB	0.63	0.46	36,101	77,896	
	WE	0.67	0.49	18,185	37,162	
Thin						−165,191
	SNB	0.92	0.72	25,980	35,943	
	WE	0.92	0.83	7800	9365	

SNB: Sentinel Node Biopsy strategy; WE: Wide Excision strategy; LYS: life years saved; QALY: quality-adjusted life years; ICER: incremental cost effectiveness ratio (SNB *versus* WE).

In cost-effectiveness terms SNB strategy was strongly dominated by WE (quadrant IV) for the first time horizons (Supplementary Fig. 1). In the latter time horizon, ICER value was found in quadrant I, so the threshold of costs to win a year of life should be defined. Thresholds proposed in the literature are 30,000€ per statistical QALY for Spain and 50,000US$ per statistical QALY for the USA, and inefficient technique thresholds 120,000€/QALY and 100,000US$/QALY respectively [25–29]. If these thresholds were taken into account, SNB was still not cost-effective compared to WE.

3.1.2. Thick CM

Over a 10-year time horizon, SNB strategy implied costs of 36,101€ per patient, and WE 18,184€. Effectiveness variables were 0.63 LYSs and 0.46 QALYs for SNB, and 0.67 and 0.49 respectively for WE. Ratios obtained for both strategies were estimated as 57,263€/LYS and 77,896€/QALY for SNB and 27,264€/LYS and 37,162€/QALY for WE (Table 3). The ICER values that compared them were −490,202€/LYS and −692,074€/QALY, which means that SNB is clearly dominated by WE (Supplementary Fig. 2). At 1 and 5 years, the results showed the same behaviour in variables and ratios, being always less favourable for SNB than for WE (Supplementary Table 2).

3.1.3. Thin CM

Similar results were obtained for this group of patients, although differences were higher than in thick CM (Table 3; Supplementary Table 2). The worst results were observed over the 10-year period, ICER values corresponding to −9,217,203€/LYS and −165,191€/QALY. At that moment, total costs per patient were 25,980€ and 7800€ respectively. In SNB 0.92 LYSs, 0.72 QALYs, 28,294€/LYS and 35,943€/QALY were estimated instead of 0.92 LYSs (slightly higher value than SNB strategy), 0.83 QALYs, 8877€/LYS and 9365€/QALY estimated for WE strategy. Graphic representation showed the results once again in quadrant IV, where the SNB strategy should be rejected because of the WE strategy dominance (Supplementary Fig. 3).

3.2. Sensitivity analyses

One-way sensitivity analyses were only performed for the comparison whose ICER results were positive, i.e.: intermediate CM over 10-year time horizon. No variability was demonstrated after the modifications of the different variables within the published ranges, SN positivity rate [30,31], 3% discount rate per year, the use of PET in follow-up [20], mortality rates in WE, SN positive and negative patients [8] and utilities [17] (Supplementary Table 3; Fig. 2).

Nevertheless, WE mortality rate provided the best ICER value for SNB, although far from the threshold of acceptance and SN positivity rate, worsened the results the most. Among all variables, those with the most influence in the results were SN positivity rate and mortality rate of SN positive, affecting less the utilities and rest of variables.

A two-way sensitivity analyses showed that ICER would be 37,572€/QALY when mortality rates in WE and in SN negative patients were adjusted together, which corresponded to the best results for SNB. With other modifications of two variables at the same time, final results did not change either. Sensitivity analyses therefore demonstrated that the model was consistent.

4. Discussion

After the cost-effectiveness study of SNB in intermediate CM performed by Morton et al., in 2009 [11], as far as we are aware, our study is the second one in this field, being the first where intermediate and thick thickness CM have been considered separately. In this period of time, MSLT-1 final results have been published for these CM thicknesses [8], which allow getting high level evidence data.

If we consider thin CM, this study would be the second one [10]. No randomized trials in thin CM have been found, MSLT-1 has a 290 patients series not published yet [8]. Probabilities were obtained from AJCC T1 CM [13] (WE strategy) and, for SNB, from top-rated article after a systematic review [15].

Model results that included the best evidence in clinical parameters, official costs and melanoma utilities showed unfavourable results for SNB technique in all CM patients. These results are opposite to those published previously [11]. Morton's conclusions demonstrated an improvement in effectiveness, LYSs and QALYs, with a mild increase in costs that located SNB strategy in the area of acceptance due of its effectiveness. Our results for different time horizons, showed the technique to fall within the worst quadrant (IV), where the indication is no acceptance of SNB. The only exception found was for intermediate CM over a 10-year horizon, but it implied costs above the published thresholds of acceptance. The main difference with the previously mentioned study is the design, based on differences between immediate and delayed CLND probabilities, ours being more focused on differences between SN involvement, and not paying attention to the time of CLND probabilities, often criticized for its post-randomization nature [32,33].

Nevertheless, for thin CM, our results are in accordance with the other cost-effectiveness study, which focused its conclusions on the high price to pay for a QALY (ranging between 518,000 and 770,000€ for a life saved) [10]. The low positivity rate used in this study, 1.4%, was questioned [11], but when the maximum value of positivity rate described was tested, 8% [34], the technique was found not to be cost-effective, its results being even worse. Our conclusions are in line with this study [10], though ours

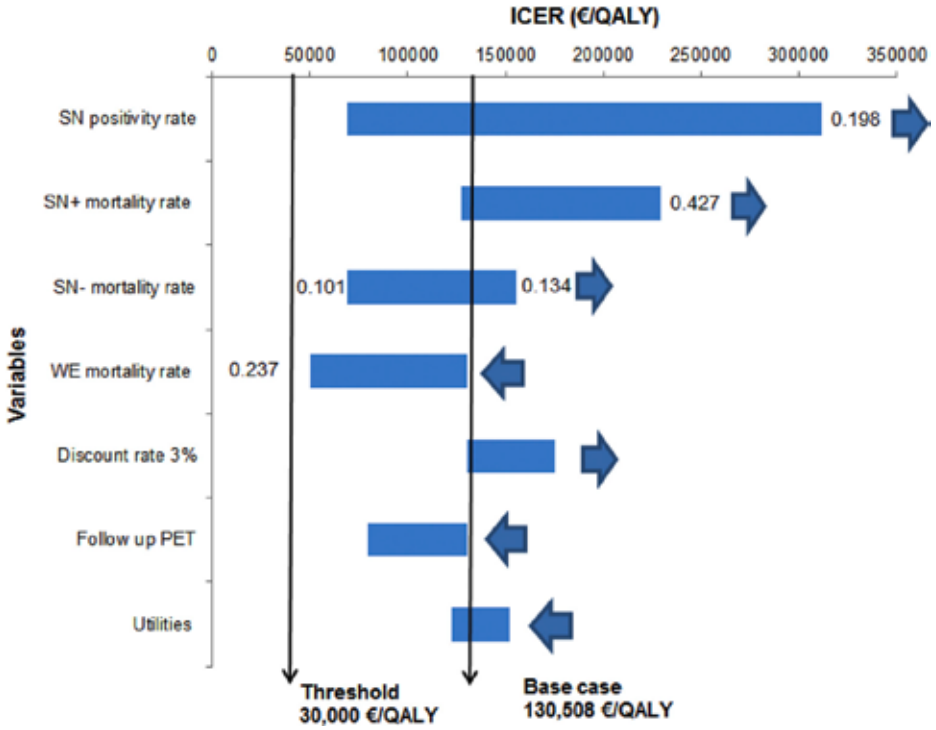

Fig. 2. One-way sensitivity analysis graph. Variation of the incremental cost effectiveness ratio (ICER) of sentinel node biopsy (SNB) *versus* wide excision (WE), depending on variable modifications. Only one variable is evaluated every time, the rest remaining constant. The ends of each bar indicate the range of ICER for each variable, and base case value and threshold ICER indicated by vertical arrows and expressed in € per quality-adjusted life year (QALY). The result of sentinel node is indicated as positive or negative as SN+ and SN-, respectively. The thick arrows show the direction of change in ICER over the specific range.

demonstrated SNB to be a less effective and more costly technique, which implies its rejection for this group of patients.

SN positivity is the variable that most influenced the variability of the model with no changes in ICER results using the published range of values [30,31]. As the number of positive SN increased, more costly results were obtained and, as it decreased, an improvement in the result was obtained, although the technique was not cost-effective. This behaviour agrees in general terms with the previous study [11], but in ours SNB did not reach the necessary cost-effectiveness for any thickness. Improvement of positivity rates is one of the most important advantages of SPECT-CT technique, if its positivity rate (25.5%) is included and the model tested, results worsened once again [35].

Mortality rate affected the final results. In fact, mortality rate of WE group 19.4% followed by mortality rate of SN negative patients 12.9%, were the parameters that, in one of their limits 23.7% and 10.1% respectively, improved the most SNB cost-effectiveness in patients with intermediate CM over a 10-year time horizon. When adjusted together, these results improved up to 37,572€/QALY. It was the best result obtained for the SNB in all groups of patients at any time horizon, but these variables are hardly modifiable, at least the first one.

Intermediate CM showed negative values for LYSs and QALYs over 1 year and over 5 years in QALYs. The slight difference in favour of SNB strategy over 10 years, represents an improvement of 0.024 QALY, 8.6 more days of life with plenty health, with much higher costs for every QALY for those patients.

For thick melanomas, differences between LYSs and QALYs were always favourable to WE strategy at any time horizon, and for thin melanomas, differences between both parameters increased over the years. In this case, since in the WE group, i.e.: the general population, 85% are still disease free and 92% alive after 10 years, the application of SNB should be questioned because patients live fewer years and their quality of life is worse.

4.1. Limitations

One of the main limitations of our study is that time-dependent disease progression data are not found in earlier literature and therefore a constant progression every year has been assumed, even though it is known that the relapse rates are higher the first 2 or 3 years, being the 85% before the 5 years [11,36]. This limitation has been overcome to some extent assuming the same relapse rates at 10 years as at 5 years for every Breslow thickness CM, no modifications in final results being obtained.

The model assumes that the options in the decision tree are mutually exclusive, in such a way that patients only receive an intervention and stay in a health state at the same time, and at least in patients with relapse, nodal and distant involvement could be overlapped at least in 12% of cases [36]. In addition, disease-free patients were obtained subtracting the other probabilities, which underestimates the value. In an essay to compensate that, the model was verified by subtracting the 12% overlapped patients to the nodal relapse group and, in the best presumption ICER values improved up to 103,598 €/QALY. Moreover, our results are Spanish costs, lower than those of USA or other European countries, which implies even worse results for the technique.

5. Conclusions

This study shows SNB is not cost-effective for CM of any thickness, since either no clinical benefit is obtained, or when a slight improvement in effectiveness is reached, it is above the economic threshold that would justify its use. These results, jointly with the lack of survival increase between SNB with lymphadenectomy for nodal metastases and observation patients, question the implementation of this technique, and its widespread use needs therefore to be reconsidered.

Funding

None reported.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animal performed by any of the authors.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.suronc.2016.05.020.

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L 35

HPV SCREENING ALGORITHMS FOR THE PREVENTION OF CERVICAL CANCER: WEIGHING BENEFIT, COST, AND BURDEN.

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Summary:

Cytology-based screening is still the most widely used approach for cervical cancer screening, but HPV-based screening recently received regulatory approval in the US and was endorsed by European Guidelines. Within the European Union, Italy, Netherlands, and Sweden are currently switching to HPV-based screening. Other countries, including Belgium, Finland, UK, are currently considering HPV-based screening. HPV-based screening can be implemented in various ways. Screening algorithms have several decision options: i) primary screening modality: HPV testing only or combination of HPV testing and cytology, ii) start and exit age, iii) screening interval, and iv) triage strategy for HPV positive women. There is no single best screening algorithm as countries differ with respect to available healthcare resources and countries weigh screening-related burden and health gains differently. However, there is general consensus that evidence about how to screen women should be based on data collected in real-life settings. Within Europe, randomized HPV-based screening trials have been conducted in Finland, Italy, the Netherlands, Sweden, and the UK and these trials provide accurate information for assessing screening-related health gains and burden. These data can subsequently be used in models for a more formal health-economic assessment.

In the first part of my presentation, I will give an overview of the European randomized HPV-based screening trials conducted in the Netherlands, Sweden, Italy, UK, and Finland and discuss how the results can be used to inform the screening interval and primary screening instrument. I will also present some cost calculations to illustrate the cost of running a program as a function of the screening interval and primary screening instrument. In the second part, I will focus on triage strategies that are currently considered and that are based on cytology, repeat HPV testing, and/or HPV genotyping. In particular, I will present results from the first two HPV-based screening rounds of the Dutch POBASCAM study. This large study (44,102 women) provides an opportunity for evaluating triage strategies as women have received HPV testing and HPV genotyping in two subsequent screening rounds. I will estimate screening-related burden and health gain by the number of colposcopies needed to find a CIN3+ case and evaluate the stability of this number over multiple HPV-based screening rounds. I will finish my talk with possible future directions for improving the efficiency of the cervical cancer screening program, including HPV self-sampling.

INVITED LECTURES

L 36

RESULTS OF EKE COS-Y, PROGRAM FOR QUALITY EVALUATION OF COMPUTER ASSISTED CERVICAL SCREENING IN FLANDERS.

dr. Bart Lelie, head of the department of pathology AZ ZENO, Knokke

In Flanders an imaging hub has been organised for years by the pathologists of Yperman Ziekenhuizen, dr. Kristof Cokelaere and dr. Stijn Deloose, unique in the world. The hub realizes the logistics for imaging according to Hologic, for 11 participating laboratories. It offers the imaging itself and if necessary also the monolayer preparation itself.

In 2016 Hologic could reduce some security restrictions which gave the opportunity to us to realize a diagnostic external quality evaluation (proficiency test). The need to do this was partially because there existed none at the moment for computer assisted screening, there was a lot to do about the "lack" of quality monitoring in pathology for the cervical screening and the opportunity that was given when Hologic gave up the unpractical CDROMS for email and USB-sticks in 2016.

As an external auditor for ISO15189 in the Netherlands for more than 7 years, dr Lelie is familiar with proficiency testing and possible (in the ISO15189 mandatory) alternative proficiency testings if none exist in the catalogue of the usual suspects as Nordiqc, CAP, AFAQAP, UKENEQAS, etc. EKE COS-Y (externe kwaliteitsevaluatie computer ondersteund screenen binnen de Ypermangroep) was born. A service level agreement (SLA) was made and signed by all participants. It stated what was required and how the EQC would work. For the administration google docs was used with forms, spreadsheets and automated mailings. We gathered a pool of Preservcyt monsters from the participants for which was stated that 66% would be NILM and 34% by biopsy proven pathologic. ASCUS-ASCH was not allowed because this group is by definition vague and is also not allowed in the diagnostic EQC from the CAP. This pool should reflect the routine screening in a laboratory of pathology.

Data was anonymised. 2 rounds followed in 2016 with randomised monolayers of good quality. We made the monolayers with a validated papanicolaou staining and our T2000. The quality was checked before sending it to the imager. The imaging itself was 100% effective. The coordinates were send by email to the participants. Round 1 had 5 slides, round 2 10 slides. The results were transmitted via google forms and processed. Each participant received a personalised report with a personal score. The scores of everyone was anonymised and given as reference. Several scoring methods were used, each with pros and cons. The policy score is based on the advice given by the pathologist on the basis of his diagnosis. This score was for us the most important one for it has direct effect on the patient's treatment.

Round 1 gave a result of ≥80% for 86.7% of the participants. Round 2 77.8%.

A symposium was organised in December 2016 to give and get feedback of the rounds, which was successful.

In 2017 we got 19 participating laboratories in Flanders and the results are coming through and will be presented as well.

References

Royal Bulletin 13-02-2014:

Praktijkrichtlijn 2014 voor de erkenning van laboratoria voor pathologische anatomie

FREE PAPERS

ORAL PRESENTATIONS

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P 01

IMMUNOHISTOCHEMICAL STUDY OF THE ATRX/DAXX/H3.3-BASED ALT PATHWAY IN PLEURAL MESOTHELIOMA

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Introduction:

Pleural malignant mesothelioma (plMM) occurs decades after asbestos exposure, suggesting the presence of, behoped drugable, telomerase (TA)-dependent or TA-independent, immortalization mechanisms. We detected TA-activity in the vast majority of plMM, in line with the frequency (15%) of TA-independent Alternative Lengthening of Telomeres (ALT) generally found in cancer, especially sarcomas. ALT de-repression relates to mutations in components of the ATRX/DAXX/H3.3 trias, resulting in nuclear ATRX/DAXX protein loss and/or presence of mutated H3.3 protein. A pathogenic link between G34R type H3.3 mutation and ALT, based on the induction of chromosomal instability by unresolved replication fork stalling was recently described.

Aim :

Interested in i) explaining low TA-enzyme activity formerly measured, ii) detecting possible TA-ALT mosaicism, and iii) testing the hypothesized ALT-sarcoma link, we studied the ALT phenotype using surrogate ATRX/DAXX/G34R H3.3 immunohistochemistry in epithelioid and sarcomatoid plMM subtypes.

Methods :

FFPE material of 22 plMM (13 epithelioid, 5 sarcomatoid, 3 biphasic, 1 desmoplastic types) was stained using anti-ATRX monoclonal antibody (Sigma clone CL0537, 1/200), anti-DAXX polyclonal antibody (Sigma, product number HPA008736, 1/200) and anti-H3.3 G34R (RevMab Biosciences clone RM240, 1/100) on a Ventana BenchMark XT autostainer. Nuclei of normal endothelium served as positive controls for ARTX/DAXX. Nuclei of the U2-OS ALT cell line and an index lungcancer case served as negative controls for ATRX and DAXX, respectively. Nuclear ATRX/DAXX loss, analyzed separately in epithelioid and sarcomatoid components, was categorized in 'ATRX/DAXX loss', 'ATRX/DAXX indeterminate' and 'ATRX/DAXX retained' groups, corresponding to signal loss in >90%, 10-90% or <10% of tumor nuclei, respectively. H3.3 G34R nuclear signal was scored absent or present.

Results :

No plMM was classified 'ATRX/DAXX loss'. 3 plMM were classified 'ATRX/DAXX indeterminate': 1 epithelioid type (classified 'ATRX indeterminate'-'DAXX retained'), 1 sarcomatoid type (classified 'ATRX indeterminate'-'DAXX indeterminate') and 1 biphasic type (classified 'ATRX indeterminate' in both components - 'DAXX retained'). The results of H3.3 G34R immunohistochemistry will be presented.

Conclusion :

We found no evidence that plMM preferentially relies on ATRX/DAXX-defined ALT. However, the 3 plMM classified 'ATRX/DAXX indeterminate' suggest at least intra-tumoral TA/ALT mosaicism in some cases. This mosaicism was seen in a proportion of sarcomatoid subtype/areas, possibly corroborating the ALT-sarcoma link. Mosaic ATRX signal loss - and thus an ALT-like phenotype – seen in overt epithelioid areas could be explained by non-phenotypically visible molecular epithelial-to-mesenchymal transition (EMT). Morphologically invisible EMT in ATRX-negative epithelioid cells should be further investigated by E-cadherin double-staining, illustrating ATRX/E-cadherin co-loss. The haphazard intermingling of ATRX negative and positive nuclei suggests an epigenetically driven loss/downregulation of function of ATRX rather than a clonal, genetic alteration. More importantly, this observed intra-tumoral mosaicism might predict resistance to anti-TA drugs. The results of H3.3 G34R immunohistochemistry in plMM will be discussed.

P 02

HISTOPATHOLOGICAL FEATURES OF METASTATIC BREAST CARCINOMAS CARRYING SOMATIC MUTATIONS IN ERBB2.

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Introduction:

Somatic human epidermal growth factor receptor 2 (ERBB2) mutations (with/without ERRB2 amplification) are expected to be a rare event in early breast cancer patients (~2%) and likely drive tumorigenesis. However, it is not fully understood if ERBB2 mutations play a role in breast cancer progression. Here, we present the first study that thoroughly investigates the demographic and clinic-pathologic features of patients with ERBB2 mutated tumors in a large unselected cohort of metastatic breast cancer (MBC) patients.

Methods:

We included retrospectively all MBC patients, independent of hormone receptor or ERBB2 amplification status and tumor availability, diagnosed between 2000 and 2015 at the Multidisciplinary Breast Center of University Hospitals Leuven. Genomic DNA extracted from the primary tumors was subjected to deep targeted re-sequencing using an assay covering 5 exons (exons 8, 17, 19, 20 and 21); mutations with allelic frequency ≥ 5% in at least one independent analysis were considered significant. Histopathology of the ERBB2-mutant tumors was centrally reviewed. We assessed the surrogate intrinsic molecular subtype by combining the protein expression level of estrogen receptor (ER), progesterone receptor (PR), ERBB2 and Ki-67 (Lum-A-like: <20%). Tumor infiltrating lymphocytes (TILs) were measured according to the guidelines of the international immuno-oncology biomarkers working group. In case of neoadjuvant treatment the pathologic response was measured.
Finally, tumor characteristics were compared between patients with and without ERBB2 mutations.

Results:

We established and validated a "research-use only" next-generation sequencing assay to identify ERBB2 mutations. ERBB2 mutations were observed in 1.8% (13/721) of MBC patients. ERBB2-mutant tumors appear to have similar patient and tumor characteristics (according to TNM stage, grade, and hormone-receptor and HER2 status) compared to non-mutant MBC patients. ERBB2 mutations occur in all molecular subtypes (6 Lum-A-like, 1 Lum-B-like, 3 Lum-HER2-like, 1 HER-2 enriched-like, 2 TNBC-like), with the highest prevalence observed in luminal-A-like tumors (6/13; 46%). ERBB2-mutant tumors were observed most commonly (69%, 9/13) in invasive ductal carcinomas, although a non-significant enrichment was observed in invasive lobular carcinomas when compared to non-mutant tumors (31% (4/13) vs. 14% (97/695), p=0.086). In ERBB2-mutant MBC no grade 1 tumors were found; 8/13 were grade 3 and 5/13 (38%) grade 2. All but one (grade 3, Lum-HER2-like) tumors had TILs score <50%, with HER2 amplified and TNBC showing the highest (10-40%) TILs scores. Only one Lum-A-like patient received neoadjuvant treatment resulting in partial response.

Conclusions:

The prevalence of ERBB2 mutations in our cohort of MBC is equal as that previously reported for non-MBC. Based on clinic-pathologic features, ERBB2-mutant tumors do not substantially differ from non-ERBB2-mutant tumors, albeit we observed a slight increase in lobular carcinomas and Lum-A-like tumors.

P 03

HIGH PD-L1 EXPRESSION IN INFLAMMATORY BREAST CANCER IS ASSOCIATED WITH B-CELL INFILTRATION

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Introduction:

Inflammatory breast carcinoma (IBC) is a rare, very aggressive form of breast carcinoma.

The tumour stroma with tumour infiltrating lymphocytes (TILs) appears to play a crucial role in the IBC phenotype.

In both IBC and nonIBC, tumours are infiltrated by TILs including B-cells, but their role in regulating anti-tumor immunity is not well understood; evidence suggests that tumour educated B-cells acquire PD-L1 expression.

Aim:

To look at the differences and outcome effects of TILS, B-cells and PD-L1-expression in the immune infiltrate of IBC and nIBC.

Methods:

TILs, CD79α+B-cells and PD-L1 were scored by 2 observers in 169 IBC and 235 nIBC tumour samples.

TIL and CD79α continuous scores (occupied area% of tumour stroma) were categorized.

PD-L1 (Clone SP142) scoring was based on the % of positive tumour cells (TC) and immune cells (IC) occupying the tumour area.

Kappa test showed substantial interobserver agreement (TIL: $\kappa = 0{,}694$, PD-L1: $\kappa = 0{,}718$, CD79α: $\kappa = 0{,}706$).

Results:

Most IBC patients presented with grade 3 (67.7%) ductal (90.5%) carcinoma. 28.9% of patients with initially localized disease achieved pathological complete response (pCR) after neo-adjuvant chemotherapy. Overall survival (OS) in the IBC cohort (5 y OS: 52,2%) was significantly shorter compared to the 5-year survival of 95,7% for the nIBC cohort ($p < 0{,}001$).

Mean TIL score was comparable between IBC (18.02%) and nIBC (19.77%), but in the hormone receptor positive (HR+) group TILs were significantly higher in IBC (15.60% vs. 11,45%; $p = 0{,}011$). CD79α infiltration of IBC and nIBC was comparable.

Only 1.8% of the IBC patients and 1.3% of the nIBC group showed PD-L1 TC positivity. PD-L1 expression in IC was significantly higher in IBC ($p < 0,001$). This difference remained significant in all subtypes, except for the HR-HER2+ subtype.

In IBC, PD-L1 expression correlated with higher intratumoral CD79α ($p= 0.029$) and TIL ($p= 0.001$) scores in multivariate analysis, but not with HR status, molecular subtype, grade or nodal status. In contrast, PD-L1 IC expression in nIBC correlated with HR- subtypes and tertiary lymphoid structures.

Univariate analysis showed that achieving pCR in IBC was significantly associated with more TIL infiltration ($p= 0.002$), PD-L1 IC expression ($p= 0.011$) and intratumoral CD79α+ cells ($p= 0.049$). However, in multivariate analysis only TIL infiltration was an independent predictor of pCR.

Survival analysis showed a significant beneficial effect of TILs, especially in the HR+ subtypes, but not for PD-L1 or CD79α+.

Conclusions:

Brisk TIL infiltration correlates with better pCR and longer OS in IBC, especially in the HR+ subtypes. CD79α+ cells and PD-L1-expression do not independently contribute to this effect.

PD-L1-expression is significantly increased in IBC compared to nIBC. This expression is associated with intratumoral CD79α+cells in IBC and might predict response to immune checkpoint inhibitors.

P 04

CASE REPORT

CASE REPORT OF AN UNUSUAL NODULE ON THE SKIN OF THE NOSE

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Content:

A 36 years old man suffered from a small, nodular and erosive lesion on the skin of his nose. Due to the clinical differential diagnosis between perifolliculitis, basal cell carcinoma and rosacea, a biopsy was taken. Microscopically, the dermis is almost entirely occupied by a poorly circumscribed proliferation, composed of confluent growing clusters and strands of epithelioid cells with large, glassy, eosinophilic cytoplasm. The nuclei show mild to moderate atypia, but mitotic figures and necrosis are absent. In the cytoplasm, small lumina are found, that are usually filled with a red blood cell, suggesting a vascular origin of the cells. According to this data, immunohistochemistry was performed. The tumour cells are positive for ERG, CD31, CD10 and CAMTA1, which is consistent with a vascular process. The pathologic differential diagnosis consists of an epithelioid angiosarcoma, an epithelioid haemangioendothelioma (EHE) and a cutaneous epithelioid angiomatous nodule (CEAN). Although atypia is present, the morphology is rather insufficient for the diagnosis of an epithelioid angiosarcoma. In addition, the atypia does not conform to a CEAN. One of the characteristics distinguishing EHE from CEAN, beside the presence of atypia, , is the circumscription of the lesion. Our lesion is poorly marginated. Considering the small size and the cutaneous location of the lesion, the preferred diagnosis was a low grade epithelioid haemangioendothelioma.

The term haemangioendothelioma is used for a variety of vascular neoplasms, having different morphology and biological behavior, ranging from benign to malignant. In the current classification of haemangioendothelioma, EHE is classified as a malignant vascular lesion. Furthermore, this lesion can be low or high grade, according to size, location and mitotic count. Low grade EHEs are usually superficial, small (<3cm) and have low mitotic activity. High grade EHEs can be located superficially or deep, are larger than 3 cm and usually have many mitoses. A follow up study on cutaneous EHE showed that 21% of the patients suffered from systemic metastasis. In another study, metastasis are mostly found in high grade EHE, while low grade EHE often follow an indolent course, although the possibility of metastasis is never completely excluded.

To our knowledge, case reports on cutaneous epithelioid haemangioendothelioma are uncommon. These cases usually end up in larger series of cutaneous vascular lesions or series of mixed deep and superficial EHE. Furthermore, a single case report on a cutaneous epithelioid angiomatous nodule mentions the similarities and differences in the differential diagnostic between CEAN and EHE.

In our case, it was important to make an accurate diagnosis, given the fact that a low grade EHE can be treated with excision and follow up, while a high grade EHE or an epithelioid angiosarcoma could result in a more aggressive surgical treatment in this young patient.

P 05

CONTRIBUTION OF PROLIFERATION AND INVASION CRITERIA TO THE PREDICTION OF RECURRENCE OF NON-FUNCTIONING PITUITARY ADENOMAS: RETROSPECTIVE ANALYSIS OF 120 CASES.

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Content:

Recently, a new classification based on precise clinical, radiological and histological criteria has been proposed, allowing the grading of pituitary tumours into 5 groups of different potential aggressiveness (Trouillas et al, 2013).

Aim :

Our study was aimed at confirming the prognostic value of this clinicopathological gradation in the prediction of aggressiveness and recurrence of pituitary tumours in an independent serie of macroadenomas which underwent first surgery in our institution.

Methods :

120 patients operated for a non-functioning pituitary macroadenoma (NFPA) were analysed retrospectively. For each of them, the invasion of the cavernous / sphenoidal sinuses by the tumour was studied on the preoperative MRI. The (atypical) proliferative character was retained if two out of three criteria were present: Ki67 ^{3} 3%, more than 2 mitoses/10 high power fields (400x) and/or positive immunoreactivity for p53.

Results :

26% (n=31) of the adenomas were proliferative and 57% (n=68) invasive. The invasive lesions were larger ($p < 0.001$) and their excision was only rarely complete (post-operative residual tumours in 82% of the cases). The distribution of NFPAs was as follows: 32% grade 1a, 11% (proliferative) grade 1b, 42% (invasive) grade 2a and 15% (proliferative and invasive) grade 2b. Their probability of recurrence within 5 years was 20, 39, 44 and 66% respectively. The young age, the atypical character and the presence of post-operative residual tumour were all independent risk factors of recurrence ($p<0.025$).

Conclusion :

The new clinicopathological classification proves to be very useful in predicting the risk of recurrence of non-functioning pituitary adenomas that have been operated. In particular, grade 2b lesions showed an overall likelihood of recurrence 8.62 times greater than those of grade 1a.

P 06

CASE REPORT

A YOUNG WOMAN WITH A SMALL CELL CARCINOMA OF THE OVARY, HYPERCALCEMIC TYPE, LARGE CELL VARIANT.

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Content:

A 30-year-old mother of 2 consulted the emergency department in July 2016 for undefinable lower back pain existing since 1 month. The pain radiated to the right fossa and was progressive since 4 days. She had no significant problems in her medical/ familial history and only used hormonal contraception as medication.

Clinical examination revealed a sensitive right fossa with a positive McBurney sign and a palpable mass. Abdominal and transvaginal ultrasound showed a uterus myomatosus with an ovarian mass of 100 x 90mm, suspected of torsion. The mass had a major solid component and was surrounded by fluid, also seen in the Douglas and hepatorenal recess. The patients CA125 was 114.8 kU/L.

A semi-urgent laparoscopy was performed, during which a bleeding, ruptured and suspicious mass was found in the right fossa. The mass was both lobulated and cystic. A classical adnexectomy was performed.

Histology showed sheets of tumor cells with a follicular-like growth pattern. The sheets were composed of large cells with eosinophilic cytoplasm (rhabdoid-like). Additionally there were large clear cells with a vesicular nucleus and a prominent nucleolus. The proliferation rate was high and there was extensive ischemic necrosis as a result of torsion. The tumor cells were EMA (partial +), WT1 (-); CD56 (focal +), Calretinin (+), Synaptophysin (+).

The tumor was diagnosed as a small cell carcinoma of the ovary, hypercalcemic type, large cell variant (SSCOHT), a very aggresive neoplasm occurring primarily in young women. The specification "hypercalcemic" is due to 2/3rd of patients have a hypercalcemia.

Subsequent PET-CT indicated bilateral para-aortal lymph node enlargement. Carboplatinum based chemotherapy was given over a period of 3 months, during which the tumor rapidly progressed, with epigastric, perirenal and iliac lymph node metastases. The patient also developed a vena cava inferior syndrome for which anti-coagulation was started. As a last resort Nivolumab was given. The patient died within 4 months of diagnosis.

POSTERS

POSTERS

POSTERS

P 07

SOLUBLE AMYLOID B-PROTEIN IS ASSOCIATED WITH INCREASING T-PATHOLOGY IN APP-TAU TRANSGENIC MICE.

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Introduction:

The extracellular deposition of the amyloid β-protein (Aβ) and the intracellular accumulation of abnormal phosphorylated τ-protein represent the pathological hallmark lesions of Alzheimer's disease (AD). Recently, it was shown that cross-seeding of τ by Aβ takes place when injecting Aβ aggregates into the brains of τ-transgenic mice.

Aim:

Here, we made use of different Aβ-producing mouse models crossed with TAU58 mice that overexpress mutant (P301S) τ driven by a Thy-1 promoter to identify the type of Aβ aggregates that interact with τ as well as to clarify whether this interaction is located within or outside the neuron.

Methods:

We crossbred τ with three different Aβ-producing-transgenic mice. At the age of six months we used immunohistochemistry to detect τ and Aβ pathology. Biochemically, we determined the Aβ-content in the soluble, dispersible, membrane-associated, and plaque-associated fractions of the brain homogenates. The content of total and phosphorylated τ-protein was determined by ELISA.

Results:

Hemizygous TAU58 mice develop compared with human AD patients low levels of neuronal τ aggregates in the form of pretangles and neurofibrillary tangles (NFTs). Crossbreeding of these TAU58 mice with APP23 mice – overexpressing human amyloid precursor protein (APP) with the Swedish mutation (670/671 KM -> NL) and producing amyloid plaques beginning with 5-7 months – leads in 6 months old animals to an increase of the percentage of τ accumulating neurons in the frontal cortex and in the hippocampus. A less strong but still detectable increase in τ pathology in hippocampal neurons was observed at this age when crossbreeding TAU58 mice with APP51/16 mice – overexpressing human wildtype APP and starting to develop plaques with 10-12 months of age. No increase of τ pathology was seen following crossbreeding with APP48 mice, in which Aβ remains intracellular. Although Aβ was produced in all three mouse lines crossbred with the TAU58 mice only those mice secreting Aβ showed

increased τ pathology. Moreover, the levels of soluble Aβ produced by 6-months-old APP23xTAU58 and APP51/16 xTAU58 mice were related to the increase of τ pathology in a dose dependent manner. Dispersible Aβ may play a less important role for interacting with τ because APP48 xTAU58 mice exhibited higher levels of dispersible Aβ aggregates than APP51/16 xTAU58 mice although the latter showed more advanced τ pathology. No correlation with τ pathology was found

for membrane and plaque-associated Aβ as well as for soluble and dispersible high molecular weight oligomers and protofibrils/fibrils.

Conclusions:

These findings strongly suggest that soluble Aβ species of low molecular weight interact with τ most likely extracellularly at the site of the synapse where τ can be released by the neurons. The interaction between τ and Aβ lead to an exaggeration of τ pathology presumably by creating more efficient seeds for the intracellular aggregation of τ in a manner that depends on the available dose of Aβ species.

Support: Alzheimer Forschung Initiative (AFI) #10810; FWO-Odysseus program.

P 08

ROUTINE HISTOPATHOLOGICAL EXAMINATION OF MASTECTOMY SPECIMENS AFTER GENDER-CONFIRMING SURGERY.

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Introduction:

The number of trans men seeking for gender-confirming surgery has steadily risen throughout the past decade. Consequently, surgical pathologists are increasingly confronted with trans male mastectomy specimens. Trans male breast tissue is still a rare specimen in surgical pathology and there are no well-defined guidelines regarding its examination.

Aim:

This study aimed to provide a detailed description of common and uncommon breast lesions in trans male breast tissue of patients treated at the University Hospital of Ghent. The utility of routine histopathological examination was investigated.

Methods:

Breast tissue of 344 trans men who underwent bilateral mastectomy at the Department of Plastic Surgery between 01/01/2005 and 12/07/2017 were included. The following data were retrieved: date of birth, age at time of bilateral mastectomy, bilateral breast weight, presence of skin in the specimen, and the number of sampled tissue blocks. All HE-stained slides were retrieved from the archives of the Department of Pathology and were systematically reviewed by two pathologists (M.V.B. and S.V.R.). In case of disagreement, a third pathologist was consulted (J.V.D., M.P. or S.V.) to obtain a consensus diagnosis. Presence of benign, premalignant and malignant breast lesions was explored. The number of terminal duct-lobule units (TDLUs) per 10 low-power fields (LPF) was quantified. The stroma/fat ratio was macroscopically assessed based upon the HE-stained slides.

Results:

Mean age of this patient cohort was 25.8 years (range 16-61 years) at the time of surgery and significantly decreased over time ($p<0.001$). Older patients presented with a significantly higher number of breast lesions and higher breast weight ($p<0.001$). The number of TDLUs per LPF was lower in heavier breasts ($p=0.006$), but did not correlate with patient age ($p=0.064$). Breast lesions, either benign or (pre)malignant, were present in 166 patients (48%). Apocrine metaplasia was seen in 24.1%, lactational changes in 3.8%, columnar cell lesions in 29.7%, sclerosing adenosis in 3.2%, fibroadenoma in 3.8%, usual

ductal hyperplasia (UDH) in 16.6% and atypical ductal hyperplasia (ADH) in 1.5%. One cavernous haemangioma was also found (0.3%). Two patients (0.6%) had an invasive breast cancer, of which one was an unexpected finding. One 31-year-old patient showed ductal carcinoma in situ (DCIS) and invasive carcinoma of no special type. A 52-year-old trans man showed DCIS, lobular carcinoma in situ (LCIS) and invasive carcinoma of no special type.

The number of breast lesions encountered upon microscopy significantly increased with the number of tissue blocks taken ($p<0.001$).

Conclusions:

This study provides a roadmap for histopathological analysis of trans male mastectomy specimens. The discovery of an unexpected breast cancer in a 31-year-old patient emphasizes the importance of thorough routine histopathological examination. We recommend that the number of tissue blocks taken should be based upon patient age and breast weight.

P 09

QUALITY ASSURANCE REPORTS ON CERVICAL SAMPLES ANALYSED IN FLEMISH PATHOLOGY LABORATORIES.

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Introduction:

The Belgian Cancer Registry (BCR) has a legal base to collect all cervical test results from the laboratories for pathological anatomy in a cervical cyto-histopathology registry (CHP). This CHP plays a crucial role in both the organization and evaluation of the Flemish cervical cancer screening program which is organized by the Agency for Care and Health from 2013 onwards. To monitor the quality of the screening program, the BCR was assigned to yearly calculate quality indicators for screening and to assure quality of the CHP.

Aim:

To improve the quality of the CHP, individual feedback reports were prepared for all Flemish laboratories to inform them on the quality and completeness of supplied data. Moreover, individual results concerning the Flemish screening program were included and, if necessary, guidelines for improvements were provided.

Methods:

The CHP is completed with reimbursement data from health insurance companies, which allows evaluating the completeness of the CHP. The two databases are linked by the social security identification number (SSN). All analyzes were performed on a defined population consisting of women with a valid SSN, residing in Flanders on January 1st of the year of sampling, and aged 25 to 64 years. Quality indicators were calculated for 52 Flemish laboratories concerning cervical samples taken in 2013, 2014 or 2015. The results were communicated to each laboratory along with the global figures. The laboratories are anonymized. Each laboratory received its identification code in order to position itself relative to the other laboratories.

Results:

- Only two laboratories do not use the recommended lesion codes.
- The completeness of the CHP for cytology gradually increased from 91% to 96%. For 46 of the 52 laboratories, the completeness is above 95% for the three years.
- 6 to 7% of the screenings are abnormal. The most frequent lesions are 'ASCU' and 'LSIL'. There is a large variation in the percentages of the different diagnoses between laboratories.

- For 13 laboratories, the ASC/SIL ratio was < 1.5 for the three years. For 9 laboratories, the ratio was very high for at least one year.
- The completeness of CHP for histology showed a slight decrease from 31% to 28%. Conisations are fairly well delivered; the registration of hysterectomies is less complete.
- HPV-triage mostly occurs on 'ASCU' and 'ASCH' lesions (90% and 87%) of which 38% and 62% are HPV positive, respectively. HPV tests are less frequent performed on 'AGLC' lesions (70%), showing 28% HPV positivity.

Conclusions:

The laboratories provide high quality data. The individual evaluations and accompanying benchmarking can still lead to quality improvement. This benefits the overall quality of the CHP and hence, these of the Flemish screening program.

P 10

TTF-1 EXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA : A CONFUSING GAME OF CLONES.

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Introduction:

Thyroid-specific transcription factor 1 (TTF-1) is a transcription factor encoded by the NKX2-1 gene. TTF-1 expression is assumed to be restricted to pulmonary and thyroid epithelium. We recently encountered a diffuse large B-cell lymphoma (DLBCL) in a man with a history of pulmonary adenocarcinoma, which showed nuclear expression of TTF-1/SPT24 without nuclear expression of TTF-1/8G7G3/1.

Aim:

This study aimed to evaluate TTF-1 expression in a large cohort of DLBCL, using two commercially available monoclonal antibodies (clones 8G7G3/1 and SPT24).

Methods:

Tissue blocks originated from 32 DLBCL patients diagnosed at the University Clinics St Luc (Brussels, Belgium), and 74 DLBCL patients diagnosed at Ghent University Hospital (Ghent, Belgium). Immunohistochemistry was performed on whole mount slides in an ISO15189 accredited lab at Ghent University Hospital. Both nuclear and cytoplasmic TTF-1 expression were evaluated. Information on immunohistochemical germinal center B-cell-like (GCB) and non-GCB subgroups according to the Hans algorithm was retrieved from histopathological reports, and was available for 93 of 106 cases (88%).

Results:

None of the DLCBL presented cytoplasmic TTF-1 expression. Nuclear TTF-1 expression was detected in four DLBCL (3,8%) by the SPT24 clone. Two of these positive cases were DLBCL of the GCB type and two cases belonged to the non-GCB subgroup. Nuclear TTF-1 staining was not observed when the 8G7G3/1 clone was applied.

Conclusions:

The 8G7G3/1 and SPT24 clones are widely used, but our study shows that the SPT24 clone might result in false positive TTF-1 expression in 3,8% of DLBCL. Although this is a rare phenomenon, it could present an important diagnostic pitfall during the investigation of poorly differentiated malignancies. To increase awareness, we recommend that this phenomenon should be included in the datasheet of the NovocastraTM liquid mouse monoclonal SPT24 antibody (Leica Biosystems, Newcastle, UK). As sensitivity and specificity of TTF-1 expression are clone-dependent, histopathologists should carefully consider which clone they use, depending on the diagnostic setting.

P 11

CASE REPORT

BEYOND APPENDICITIS : 2 CASES OF UNEXPECTED FINDINGS IN APPENDECTOMY SPECIMEN.

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Content:

Our first case was a 28-year-old woman in her 26th week of an uncomplicated pregnancy who presented with right sided abdominal pain and nausea. Physical examination showed rebound tenderness. A pelvic sonogram was performed and showed an intrauterine pregnancy. The appendix was not visible. The clinical diagnosis of acute appendicitis was made and the patient underwent laparotomy. Microscopic examination revealed an inflamed appendix with peritonitis on transmural endometriosis with decidual reaction.

Our second case was a 52-year-old man with adenocarcinoma of the lung known for 4 months. PET CT revealed abnormal abdominal uptake suspected for necrotic adenopathy. During laparotomy, the surgeon resected the appendix because of its inflammatory and swollen appearance. Microscopic examination revealed appendiceal metastasis from adenocarcinoma of the lung, the first known metastasis in this patient.

Appendectomy is one of the most frequently performed operations worldwide. 77 to 94% appendectomy specimens are positive for acute appendicitis on histopathologic examination. Other specimens are normal appendices (extra-appendiceal pathologies) or unexpected pathologies, as these two cases. The incidence of endometriosis occurring on appendectomies is less than 1 in 1000. Only few cases of appendiceal metastases form lung cancer have been previously reported.

These two cases emphasize the importance of histopathological examination of the appendix.

P 12

CASE REPORT

FOAMY CHANGES IN PLACENTAL TROPHOBLAST: A CLUE TO THE DIAGNOSIS OF LYSOSOMAL STORAGE DISEASE.

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Content :

Introduction

Lysosomal storage diseases are inherited disorders causing cell changes due to excessive accumulation of macromolecules in lysosomes caused by a defective or absent enzyme. This is seen in diverse organs, including the placenta.

Here, we present a case with striking foamy cytoplasmic change of villus trophoblast and stromal cells. This was a clue to the diagnosis of a fetal storage disorder, in this case a galactosialidosis.

Case report

The patient was the third baby conceived by consanguineal parents. He showed prenatal dysmorphic facial features (retrognathia, protruding ears), a sandal gap and a club foot. There was also polyhydamnios, subcutaneous edema, hydrocele and ascites, but the parents did not want any further prenatal testing. They have two healthy girls. The mother had a number of miscarriages.

The baby was born at term. In addition to the prenatally detected features, there were petechia, telangiectasias and a simian crease,

In the blood there were vacuolated lymphocytes. There was thrombocytopenia, hypoalbuminemia and elevated liver enzymes (alkaline phosphatase, gamma-GT). The urine showed proteinuria and oligosaccharidurie. Results of other standard blood tests were within normal limits.

The babygram showed osteopenia, a rough trabecular aspect of the bones and metaphyseal cupping and fraying. On ultrasound, an enlarged liver and spleen were noticed. These changes are compatible with a metabolic disease.

Examination of the placenta showed a rather large disc (95th percentile). The chorionic villi were lined by vacuolated cyto- and syncytiotrophoblast resulting in strikingly large, very pale cells. The foamy, vacuolated aspect was also seen in the stromal cells of the villi. These findings were indicative for a lysosomal storage disease.

The vacuolated lymphocytes, hepatosplenomegaly, the huge hydrocele and especially the oligosaccharidiuria pointed in the direction of infantile sialidosis or galactosialidosis with homozygosity mapping favoring the latter. Mutation analysis is still ongoing.

The baby was given best supportive care and died at the age of 5 weeks 4 days. Autopsy was refused by the parents for religious reasons.

Conclusion

With the presentation of this case, we want to point out an exceptional case showing that pathological placental findings can be an important clue in the early detection of a metabolic disorder.

Since vacuolated trophoblast can be seen in various storage diseases, enzyme analysis and genetic testing is needed for diagnosis and further management.

P 13

CASE REPORT

CASE REPORT: AN UNCOMMON TUMOR WITH AN UNUSUAL PRESENTATION.

G. Broeckx (1), M. Lammens (1), P. Pauwels (2), L. Yperzeele (1), B. Paelinck (1), O. D'Archambeau (1), V. Siozopoulou (1) / [1] Universitair Ziekenhuis Antwerpen, Edegem; [2] Working group EQA, Commission of anatomic pathology, 1050 Brussels

Content :

A 34 years old male patient presented at the emergency department with migraine and left hemiparesis. The neurologic examination showed an ischemic stroke (CVA), due to a thrombus at the level of the arteria cerebri media segment M1 right. The patient underwent a thrombectomy with removal of a gelatinous/white thrombus. A cardiovascular advice was asked and transthoracic ultrasound revealed a mass in the interatrial septum. Given the localization and the presentation, a myxoma of the heart was clinically suspected. The pathology of the thrombus material demonstrated tumoral tissue fragments, mostly consisted of cystic spaces lined by a layer of cuboidal epithelioid cells with minimal atypia. Mitotic activity was also not apparent. The tumor cells were positive with broad spectrum keratin and EMA. Partial staining for synaptophysin and CD56 was noticed. The histology was indicative of a cystic tumor of the atrioventricular node. In the differential diagnosis, metastasis from a carcinoid or another neuroendocrine tumor was also suggested. Meanwhile, additional radiological examination revealed also kidney and spleen infarcts suggestive for tumor emboli.

The patient underwent excision of his heart lesion. Microscopic examination of the excision specimen showed tumor tissue with extensive necrosis and calcification. The vital tissue consisted of an epithelial component, as already was seen in the thrombus material, but also of a stromal component, with spindle cells with minor atypia. Mitotic activity was here more pronounced. This image raised the suspicion of a malignant tumor. Given the biphasic morphology, molecular research was performed and revealed an SS18 translocation. The final diagnosis was a biphasic synovial sarcoma (SyS) of the heart with emboli in the brain, kidney and spleen.

Synovial sarcoma are rare malignant neoplasms of mesenchymal origin, that comprises less than 1% of all adult malignancies. The majority of the tumors (~70%) arise in the deep soft tissue, but localization in unusual anatomic sites, such as mediastinum, has also been described. Histologically, a SyS can be monophasic or biphasic. A monophasic consists mostly of fairly uniform spindle cells arranged in sheets or fascicles or having a herring-bone pattern. In biphasic pattern there is moreover an epithelioid component arranged in nests or in glands. Poorly differentiated areas can be a component of a monophasic or biphasic SyS. Rarely a SyS is entirely poorly differentiated. Although there is not a pathognomonic immunhistochemic profile, more than 95% of the tumors is characterized by the t(X;18)(p11;q11) translocation, which is found exclusively in this tumors.

To our knowledge, we demonstrate an exceptional presentation of synovial sarcoma. Moreover, there is not much known in the literature about the existence of neuroendocrine features in a SyS, as we show in this case.

P 14

CASE REPORT

MALIGNANT RHABDOID TUMOUR: A CASE REPORT.

K. Wilgenhof, M. van den Akker, R. Forsyth, A. Goossens, P. Lefesvre / UZ Brussel, Brussel

Introduction:

Malignant rhabdoid tumour (MRT) is a rare tumour, which typically presents in infancy. Mortality rates are high and there's often local recurrence or metastasis to lymph nodes or lungs. The tumour mostly occurs in the kidney and the central nervous system (ATRT). The exact origin of the neoplastic cells remains unknown. The population typically shows rhaboid features, but histology may be variable. Here we present a case of an extra-renal malignant rhabdoid tumour.

Case Report:

A one year old male infant with a history of chronic constipation and alternating abdominal pain was admitted to the hospital for acute intestinal obstruction. The abdominal MRI showed a large abdominal mass (5.6 cm) compressing the rectum. Colonoscopy revealed a haemorrhagic and necrotic mass bulging in the lumen. Histological analysis showed a highly mitotic and vascularised epithelioid neoplasia composed of cohesive cells with slightly eosinophilic cytoplasm and vesicular nuclei with a prominent nucleolus. There were no typical rhabdoid features to be observed. However, immunohistochemistry was typical with a strong EMA positivity, focal keratin (AE1/3) positivity and an complete loss of IN1 expression. SALL4 was positive, and all other germinal cell markers were negative (Glypican3, β-HCG, OCT3a and αFP). Additional imaging studies showed mediastinal and hilar lymph node metastasis and a solitary lung metastasis. The final diagnosis was concluded to be an extra-renal malignant rhaboid tumour. A combined chemotherapy was initiated, and induced a discrete reduction of the abdominal mass within a few weeks.

Discussion:

In infants, INI-1 immunohistochemistry can be used to exclude other malignant tumours that might show some rhabdoid features. However, in adults the loss of expression of INI1 has been observed in various other tumours. A difficult differential diagnosis can happen between MRT and an epitheloid sarcoma (ES), since they both might show rhabdoid features and both have a loss of INI-1 expression.

SALL4 is a zinc-finger transcription factor that is a key member of the complex transcriptional regulatory network of embryonic pluripotency. Different studies showed that SALL4 could be a useful biomarker in the differential diagnosis between ES (SALL4 –) and MRT (SALL4 +).

INI1 is expressed in all normal cells and is encoded by the SMARCB1 gene located at the 22q11 locus. INI1 loss is a consequence of a bi-allelic inactivation of SMARCB1. SMARCB1/INI1 is a member of the SWI/SNF chromatin-remodelling complex and plays a critical role in epigenetic regulation affecting cell cycle progression and crosstalk between cell cycling cascades. Surprisingly these

aggressive and fast growing cancers are among the most genomically stable, with most tumours showing a simple karyotype. It is thought that the main oncogenic driver is a deregulation of epigenetic homeostasis, affecting the whole genome.

P 15

CASE REPORT

COLORECTAL MUCOSAL SCHWANN CELL "HAMARTOMA": A RARE AND UNDER-RECOGNIZED ENTITY.

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Introduction:

Colorectal benign neural proliferations may present as masses or more commonly as small polyps. Some of these tumors shows association with inherited syndromes, mainly type 1 neurofibromatisis (NF-1) and multiple endocrine neoplasia, type 2 (MEN 2B). A new group of mucosal neural polyps, not associated with inherited syndromes, is purely composed of S-100 positive Schwann cells and designated as "mucosal schwann cell "hamartoma"" by Gibson and Hornick in 2009.

Objectives:

To describe the clinical, endoscopic, histopathological and immunohistochemical findings in a mucosal schwann cell "hamartoma" of the rectum.

Case summary:

A 25-mm-sized rectal lesion was found in a follow-up colonoscopy of an asymptomatic 65-year-old female patient who has no history of other neural lesions or inherited syndromes but a history of colorectal adenomas (low grade dysplasia) resected in 2007 and 2010. Endoscopically, the lesion was depressed. A biopsy sample showed on histopathological examination a poorly circumscribed proliferation of spindle cells located in the lamina propria that entrapped the colonic crypts. The cells were uniform and showed elongated, wavy nuclei, abundant eosinophilic cytoplasm and indistinct cell borders. No mitotic activity was observed. Immunohistochemically, cells showed reactivity for S-100 protein, SOX-10 and calretinin. Other markers evaluated, including smooth-muscle-actin, caldesmon, CD34, desmin, DOG-1 and C-KIT were negative. A diagnosis of mucosal schwann cell "hamartoma" was made.

Conclusion:

Only thirty-six cases of mucosal schwann cell "hamartoma" were reported in the literature. All lesions were described as polyps, mainly sessile. Herein we describe an unusual presentation of a mucosal schwann cell "hamartoma" as an excavated rectal lesion. As

in our case, literature shows a slight female predominance and predominance in the rectosigmoid colon. While lesion in our case measured 25 mm, reported lesions measured no more than 10 mm. Main differential diagnosis includes gastro-intestinal stromal tumors (GIST), solitary rectal ulcer, leiomyoma, neurofibromas, neuromas, ganglioneuromas, schwannomas, perineuromas, mucosal benign epithelioid nerve sheath tumor and inflammatory fibroid polyps. An immunohistochemical panel of S100, Caldesmon, EMA, CD34, C-KIT, and neurofilaments along with some characteristic clinical and histopathological criteria help distinguish these entities. Diagnosis of mucosal schwann cell "hamartoma" remains one of exclusion. Mucosal schwann cell "hamartoma" is a pure S-100-positive spindle shwann cell proliferation located in the lamina propria. The clinical course is benign but an underlying submucosal nodule or mass should be excluded endoscopically.

P 16

DOES PRE-IMPLANTATION BIOPSY PREDICT OUTCOME AFTER KIDNEY TRANSPLANTATION FROM LIVING DONOR?

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Introduction:

Time-zero biopsy is used as a valuable decision-making tool for renal transplantation. In contrast to data available from studies of cadaveric donors, pathological criteria were less reported in pre-implantation kidney biopsy from living donor.

Aim :

The aim of this study was to identify subclinical pathological findings on pre-implantation biopsies from living donor and to correlate these findings with graft outcome.

Methods :

We evaluated 74 living donor biopsies performed at Cliniques Universitaires Saint-Luc between October 2005 and March 2015. Time-zero biopsies were evaluated using the Remuzzi score and the Banff histopathological consensus criteria for preimplantation kidney biopsies.

Results :

According to Remuzzi score, global glomerulosclerosis, tubular atrophy, interstitial fibrosis and vascular fibrous intimal thickening were mostly absent or of mild degree. Using the Banff score, interstitial fibrosis, tubular atrophy, interstitial inflammation, arterial intimal fibrosis, and arteriolar hyalinosis were also mostly absent or of mild degree. Glomerular thrombi, focal segmental glomerulosclerosis, nodular glomerulosclerosis and tumor were absent. Acute tubular injury/necrosis of mild degree was observed in 89% (66/74) of time-zero biopsies. Global glomerulosclerosis was observed in 73% (54/74) of cases. Mean percentage of global glomerulosclerosis was 9.65% (median: 5.1%; range: 0 - 68.7%). Mesangial IgA nephritis was incidentally discovered in 7% (5/74) of biopsies. On univariate analysis global glomerulosclerosis has been shown associated with higher recipient serum creatinine values at day 10, 1 year and 2 years after transplantation (p=0.01, 0.03, 0.03, respectively). Acute tubular injury/necrosis (ATI/N) was also associated with higher recipient serum creatinine values at 1 year, 3 years and up to 6 years after transplantation (p= 0.03, 0.004, 0.02, 0.04, 0.03, respectively). Global glomerulosclerosis correlates with donor age (p=0.0019). Using a repeated-measures mixed model, ATI/N and global

glomerulosclerosis were selected as fixed effects and donor age as random effect. Global glomerulosclerosis (more than 5%) and ATI/N of mild degree were correlated with worst graft outcome over a period of 6 years (p=0.0448, p=0.0295, respectively).

Conclusion :

The results of this study demonstrate the impact of donor age, percentage of global glomerulosclerosis and of ATI/N on early and late graft function.

P 17

CASE REPORT

A RECTAL UNDIFFERENTIATED SPINDLE AND ROUND CELL NEOPLASM WITH PARAGANGLIOMA-LIKE FEATURES.

H. Nassereddine (1,2), L. Libbrecht (3), R. Sciot (4), D. Leonard (2), J. Libert (2), M. Debiec-Rychter (4), P. Baldin (2), A. Jouret-Mourin (5) / [1] Department of Pathology, Cliniques Universitaires Saint Luc,; [2] Cliniques Universitaires Saint Luc, Woluwe Saint Lambert; [3] CUSL, Brussels; [4] University Hospitals KU Leuven, Leuven; [5] Cliniques Universitaires Saint-Luc, Bruxelles

Introduction:

The (peri)rectal region can be affected by a variety of tumor(-like) conditions, mainly adenocarcinoma.

Objectives:

To describe the clinical, histopathological, immunohistochemical and molecular findings of an undifferentiated rectal mass.

Case summary:

A 13-year-old male presented with rectal bleeding and abdominal pain since one year. Colonoscopy revealed a polypoid ulcerated mass with well-defined margins located in the rectum. MRI showed a 6.9 cm pelvic mass stable after 6 months of follow-up. A PET- scan was negative. The biopsy was interpreted as an undifferentiated tumor consistent with paraganglioma. The mass was resected using a trans-anal minimally invasive surgery was and appeared polypoid, whitish and measured 6 x 5 cm. Microscopic evaluation showed an intramural ulcerated tumor composed of sheets and organoid nests of rounded and spindle cells, surrounded by a rich vascular network; some of these aspects were reminiscent of "Zellballen". Nuclei were vesicular and atypical but there was minimal pleomorphism, cytoplasm was scanty and mitotic activity was low (1/10 high power field). Immunohistochemical analysis showed positivity for CD56 and neuron-specific enolase and the tumor was completely negative for chromogranin A, synaptophysin, pancytokeratin, CAM5.2, desmin, SOX10, CD99, ETV4, EMA, SALL4, CD34, SF-1, inhibin, WT-1, PU.1, HMB45, Melan-A, STAT-6, myogenin, smooth-muscle actin, DOG1, CD117 and GATA-3. TFE-3 immunostaining was of low-moderate intensity and heterogeneous. In the areas with organoid nests, S100 protein immunostaining highlighted the presence of cells located at the periphery of the nests, giving the impression of sustentacular cells. Ki-67 positivity was estimated at 10%. SDHA and SDHB were diffusely positive. FISH break-apart analysis was negative for SS18 EWSR1, BCOR and CIC. A regional lymph node was normal. The patient received no adjuvant therapy after surgery. The patient is well at 4 months of follow-up.

Conclusions:

Rectal tumors can be mucosal, submucosal and/or mural in origin. The former consist of epithelial polyps, adenocarcinomas, MALT lymphomas, melanomas and neuroendocrine tumors. The latter include GISTs, deep leiomyomas, leiomyosarcomas, duplication cysts and hemangiomas. Some masses originate in perirectal tissue, mainly congenital and neurogenic tumor and tumor-like conditions. Paraganglioma is a non-epithelial neuroendocrine tumor which can develop in ectopic sites and is extremely rare in the rectum with two cases recorded. Microscopically, other tumors that may enter into the differential diagnosis include PEComas, alveolar soft part sarcomas, rhabdomyosarcomas, Ewing sarcomas, BCOR-CCNB3 round cell sarcomas, CIC-rearranged sarcomas, synovial sarcomas,

desmoplastic small round cell tumors, carcinomas, neuroblastomas and solitary fibrous tumors. In our case some aspects were reminiscent of paraganglioma, but the immunohistochemical and molecular analysis showed no evidence of a specific line of differentiation. In summary, we presented a case of rectal undifferentiated spindle and round cell neoplasm with paraganglioma-like features. The patient remained well 4 months after surgery

P 18

INDOLEAMINE 2, 3-DIOXYGENASE EXPRESSION IN COLORECTAL CANCER ACCORDING TO TNM AND MICROSATELLITE STATUS.

A. Meireson (1), I. Chevolet (1), E. Hulstaert (1), V. Kruse (1), K. Geboes (1), L. Ferdinande (1), P. Demetter (2), L. Brochez (1) / [1] University Hospital Ghent, 9000 Gent; [2] Hôpital Erasme, Bruxelles

Introduction:

Targeting immune check point molecules has become a major new strategy in the treatment of several cancers. Indoleamine 2, 3-dioxygenase (IDO) inhibition might become one of the next generation immunotherapies.

IDO is an intracellular immunosuppressive enzyme and its expression/activity has been reported in several cancers. We reported early expression of IDO in melanoma, remaining consistent in metastatic disease.

Aim :

The aim of this study was to investigate whether the same expression pattern could be observed in colorectal cancer (CRC). Microsatellite stable (MSS) CRCs were compared to microsatellite instable (MSI-H) CRCs, as these tumors are considered to have different immunogenicity.

Methods :

In a cohort of 78 CRC patients, primary tumors (PTs) with corresponding tumor-draining lymph nodes (TDLNs, n=38) and metastases (n=19) were retrospectively analyzed by immunohistochemical staining for IDO, CD8 and Foxp3.

Results :

Similar to melanoma a highly consistent expression pattern of IDO was observed in the PT, TDLNs and metastases. IDO was expressed both by tumoral cells and host endothelial cells and in CRC these expressions were highly correlated ($p<0.001$). Tumoral IDO expression was increased with T stage of the primary tumor. IDO positivity in the TDLNs was correlated with the presence of Foxp3+ regulatory T cells; there was no correlation with CD8+ TILs. IDO expression was significantly higher in CRCs with MSI compared to MSS (resp. 50% vs 24% for tumoral expression ($p=0.025$) and resp. 44% vs 15% for endothelial expression ($p=0.009$)).

Conclusion :

IDO expression is highly consistent in the PT, TDLN and metastatic tissue of patients with colorectal cancer, indicating that immune resistance may be determined very early in the disease course. In this way IDO could be a marker that could be of value in the immunoscore of tumours. MSI-H tumors had higher IDO expression by tumoral and endothelial cells compared to MSS tumors, implicating potential benefit of anti-IDO therapeutic strategies in this specific genetic subset of CRC.

P 19

CASE REPORT

VAGINAL METASTASIS OF RENAL CLEAR CELL CARCINOMA: A CASE REPORT AND REVIEW OF LITERATURE.

S. Bouri, V. Bogne, JC. Noël, L. Verset / Erasme Hospital (ULB), Brussels

Content:

A 76-year-old woman was referred to gynecological consultation because of metrorrhagia. In her previous medical history, we noticed one year before a left nephrectomy for renal clear cell carcinoma with sarcomatoid differentiation, Furhman grade 4, staged pT3aN1 and associated with synchronous liver metastases.

Colposcopic examination revealed the presence of vaginal ulcerated budding lesion located on the anterior wall. This lesion was treated by excision. Histopathological assessment showed highly pleiomorphic cells with enlarged nuclei scattered in a vascular stroma; these neoplastic cells exhibit positivity for vimentin, PAX8 and CD10 confirming the diagnosis of vaginal metastasis of renal clear cell carcinoma.

Vaginal metastasis is a rare entity. In the literature, colorectal, renal, bladder and uterine cancer are the main origins of such metastases. This diagnosis should be considered in every patient with previous oncological history presenting vaginal bleeding.

P 20

CASE REPORT

IGG4-RELATED INFLAMMATORY PSEUDOTUMOUR AFTER RADICAL PROSTATECTOMY : A CASE REPORT.

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CUB ULB Hopital Erasme, Bruxelles

Content:

A 74-year-old man was referred for a paracolic mass discovered one year after a radical prostatectomy for prostatic adenocarcinoma. The abdominal computed tomography showed an extensive pelvic mass pushing downward and forward the sigmoid and bladder inducing a bilateral ureterohydronephrosis. PET-scan revealed intensely hypermetabolic activity within the mass. A biopsy was carried out but the obtained material was not sufficient to allow a diagnosis. Then, the mass was surgically removed.

Macroscopically, the mass was well-defined, adjacent to the colon and presented a yellowish to greenish color.

Microscopically, we observed a proliferation of spindle cells associated with a dense inflammatory infiltrate composed of polymorphonuclear neutrophils, lymphocytes and plasmocytes. CD138 immunostaining confirmed the presence of numerous plasmocytes which were positive for IgG4 immunostaining (more than 50 IgG4 positive plasmocytes per high power field). Regarding these histopathological characteristics, we proposed the diagnosis of IgG4 inflammatory pseudotumour.

Immunoglobulin G4-related disease (IgG4-RD) is a regional or systemic fibro-inflammatory disease of unknown etiology. A closer understanding of the role of T cells and B cells in the increased production of IgG4 has led to a notion that IgG4 can act as a pathogen or an anti-inflammatory agent. IgG4-RD remains often underdiagnosed due to unfamiliarity by clinicians; moreover, a challenging multitude of clinical manifestations makes the diagnosis cumbersome. Distinctive histopathological features such as dense lymphoplasmacytic infiltrates with abundant IgG4-positive plasma cells, storiform fibrosis and obliterative phlebitis with inflammatory swelling or development of tumefactive lesions can be observed. Treatment consists in glucocorticoid therapy; this treatment should be administrated especially when vital organs are involved.

P 21

CASE REPORT

MALIGNANT RHABDOID TUMOUR: A CASE REPORT.

K. Wilgenhof, M. van den Akker, R. Forsyth, A. Goossens, P. Lefesvre / UZ Brussel, Brussel

Introduction:

Malignant rhabdoid tumour (MRT) is a rare tumour, which typically presents in infancy. Mortality rates are high and there's often local recurrence or metastasis to lymph nodes or lungs. The tumour mostly occurs in the kidney and the central nervous system (ATRT). The exact origin of the neoplastic cells remains unknown. The population typically shows rhaboid features, but histology may be variable. Here we present a case of an extra-renal malignant rhabdoid tumour.

Case Report:

A one year old male infant with a history of chronic constipation and alternating abdominal pain was admitted to the hospital for acute intestinal obstruction. The abdominal MRI showed a large abdominal mass (5.6 cm) compressing the rectum. Colonoscopy revealed a haemorrhagic and necrotic mass bulging in the lumen. Histological analysis showed a highly mitotic and vascularised epithelioid neoplasia composed of cohesive cells with slightly eosinophilic cytoplasm and vesicular nuclei with a prominent nucleolus. There were no typical rhabdoid features to be observed. However, immunohistochemistry was typical with a strong EMA positivity, focal keratin (AE1/3) positivity and an complete loss of IN1 expression. SALL4 was positive, and all other germinal cell markers were negative (Glypican3, β-HCG, OCT3a and αFP). Additional imaging studies showed mediastinal and hilar lymph node metastasis and a solitary lung metastasis. The final diagnosis was concluded to be an extra-renal malignant rhaboid tumour. A combined chemotherapy was initiated, and induced a discrete reduction of the abdominal mass within a few weeks.

Discussion:

In infants, INI-1 immunohistochemistry can be used to exclude other malignant tumours that might show some rhabdoid features. However, in adults the loss of expression of INI1 has been observed in various other tumours. A difficult differential diagnosis can happen between MRT and an epitheloid sarcoma (ES), since they both might show rhabdoid features and both have a loss of INI-1 expression.

SALL4 is a zinc-finger transcription factor that is a key member of the complex transcriptional regulatory network of embryonic pluripotency. Different studies showed that SALL4 could be a useful biomarker in the differential diagnosis between ES (SALL4 –) and MRT (SALL4 +).

POSTERS

INI1 is expressed in all normal cells and is encoded by the SMARCB1 gene located at the 22q11 locus. INI1 loss is a consequence of a bi-allelic inactivation of SMARCB1. SMARCB1/INI1 is a member of the SWI/SNF chromatin-remodelling

complex and plays a critical role in epigenetic regulation affecting cell cycle progression and crosstalk between cell cycling cascades. Surprisingly these aggressive and fast growing cancers are among the most genomically stable, with most tumours showing a simple karyotype. It is thought that the main oncogenic driver is a deregulation of epigenetic homeostasis, affecting the whole genome.

P 22

HANDLING GENOMIC BIG DATA FROM A ROUTINELY USED NEXT GENERATION SEQUENCING.

M. Rassy, I. Laios, A. Antoniou, M. Gustin, T. Stamopulos, L. Craciun, R. De Wind, M. Chintinne, D. Larsimont, T. Sticca / Institut Jules Bordet, Bruxelles

Introduction:

Next Generation Sequencing (NGS) produces important amount of data. Software are often used to filter the data and to help molecular biologists and pathologists reduce the amount of reported variants.

Aim:

We hereby wanted to widen the search for most probably pathogenic variants, beyond the actionable mutations routinely reported.

Methods :

We have analyzed all variants from all patients investigated from the 1st of January 2016 to the 30th of June 2017 on the Illumina Miseq® sequencer, using the 212-amplicon cancer panel targeting 48 genes. Cases were considered eligible for analysis when they satisfied all the following conditions: tumor cell percentage greater or equal to 10%, extracted DNA yield greater or equal to 12.5 ng, mean coverage greater than 100 reads and FFPE Quality Control (QC) score less or equal to 4.

Results :

From the 512 samples analyzed by NGS, 503 had an eligible test. The mean tumor cell percentage was 50.5%. The mean extracted DNA yield before sequencing was 2198.1 ng. The mean coverage per case was 6974.5 reads. After sequencing, 72076 DNA variants were found in the 503 eligible cases. Those were separated into 48799 supposed neutral variants (intronic, noncoding, synonymous or silent - 67.7%) and 23277 supposed modifying variants (amino acid deletion, amino acid insertion, frameshift, missense, nonsense, splicing or unknown - 32.3%). Of the 23277 supposed modifying variants, only 9692 variants (41.6%) had a coverage greater or equal to 100 reads, an allelic frequency greater or equal to 2.5% and a minimal Phred Genotype Quality (GQX) greater or equal to 30. After the matching with the EXAC database, 630 variants were known polymorphisms (with a reported frequency in the general population greater or equal to 1.0%), accounting for 6.5% of our supposed modifying variants. After excluding the polymorphisms, we compared to the COSMIC database (hg19 and COSMIC v81). On one hand, we found 2818 matching single nucleotide variants (SNV) (31.2%): 2474 missense mutations, 324 nonsense mutations and 20 unknown mutations. After applying the FATHMM score, those

were reduced to 2548 SNV: 2236 missense mutations, 292 nonsense mutations and 20 unknown mutations. On the other hand, to those SNV, we added all the 1750 nonreported variants that however affect the number of nucleic acids (mainly 1258 frameshifts and 70 non-frameshift deletions/insertions). Overall, 4298

most probably pathogenic variants were found in the 72076 DNA variants in 503 cases, averaging 8.5 most probably pathogenic variants per case.

Conclusion :

Setting wide filter criteria showed that 6.0% of the big data generated by NGS is most probably pathogenic. It exceeds the usual amount of reported mutations in pathology and can explain the discrepancies in treatment outcomes.

P 23

BAP1 IMMUNOSTAINING IN MESOTHELIAL PATHOLOGY BELGIAN MESOTHELIOMA REGISTRY

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Introduction:

BAP1 protein is the abbrevation of BRCA1- associated protein1. BRCA 1 gene is localised on chromosome 17q. A mutation of this gene is believed to account for 45% of families with increased incidence of early- onset breast and ovarian cancer.

BAP1 gene is a true TSG and germline BAP1 mutations are associated with an autosomal dominant tumour syndrome: uveal melanoma, RCC, mesothelioma in young patients. It encodes a protein of 1853 aminoacids.

BAP1 protein and BARD1 (BRCA1 associated Ring Domain) protein are both mediators of tumor suppression: double hit BAP1 inactivation can be detected by immunohistochemistry.

Staining for BAP1 protein is a highly specific marker in the differentiation of epithelioid mesothelioma from reactive mesothelial proliferations. (Cigognetti et al 2015), in effusion cytology specimens (Hwang et al 2016).

Aim :

Since 2 years the BAP1 immunostaining is added to the applied panel for the diagnosis of mesothelioma.

In order to facilitate the distinction of mesothelial cell proliferation amid the often severely inflamed background.

Methods :

A double staining of BAP1 protein/ Cytokeratin 5 is used.

Results :

The BAP1 immunostaining revealed in 89 epitheliod mesotheliomas loss of the BAP1 protein in 66 % of the cases. In 16 cases of biphasic mesothelioma there was a loss othe BAP1 protein in 12.5 %. In 9 sarcomatoid mesotheliomas a loss of the protein was observed in 11 %.

The relevance of the BAP1 immunostaining was very useful in effusion cytology samples: all 4 pleural cytology samples demonstrated loss of the BAP 1 protein

which in combination with the confirmation by immunostaining of mesothelial cells allows to make the diagnosis of malignant mesothelioma.

Another very important application of the BAP1 immunostaining refers to atypical mesothelial cell reaction in a background of reactive pleural changes altered by chronic inflammation. 8 cases demonstrated in 62% a loss of the BAP1 protein allowing the diagnosis of malignancy. In absence of invasive growth the diagnosis of atypical mesothelial hyperplasia or mesothelioma- in situ is made.

All fibrous pleurisy cases (n12) demonstrated presence of the BAP1 protein.

Conclusion :

BAP1 protein immunohistochemistry is to recommend in conditions of atypical pleural effusions and atypical mesothelial cel hyperplasia.

P 24

MISMATCH REPAIR DEFICIENT COLORECTAL CANCER: COMPARISON OF AN IVORY COAST WITH A BELGIAN COHORT.

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Introduction:

According to European and American series, up to 20 % of colorectal cancers are characterised by instability at microsatellite sites and have deleterious mutations in mismatch repair (MMR) genes (MLH1, MSH2, MSH6 and PMS2) or hypermethylation of the MLH1 promotor gene. MMR deficient colorectal cancers are predominantly found in the right colon.

Although an increasing rate of colorectal cancer has been observed in many low- and middle-income countries including in West-Africa, data on epidemiology and biology of colorectal cancer in native Africans from this region are scarce.

Aim :

We aimed to study the prevalence of MMR deficiency in Ivory Coast and to compare the data with those from a tertiary center in Belgium.

Methods :

Immunohistochemistry for MLH-1, MSH-2, MSH-6 and PMS-2 was performed on paraffin-embedded tissue samples from 83 colorectal cancers (54% males) operated in Abidjan and from 343 colorectal cancers (48% males) from Brussels. Immunohistochemical staining was interpreted as normal or loss of expression.

Results :

Colorectal cancer is occurring at a younger age in Ivory Coast compared to Belgium (median age: 53 vs. 66; $p<0.05$). In both populations, MMR deficiency was detected in 13% of cases (11 and 43 cases, respectively). Whereas MMR deficient cancers in Brussels were mainly found in women (26/43 i.e. 61%), only 3/11 (27%) of the MMR deficient cancers from the Abidjan series occurred in female patients. Moreover, the predominant location of MMR deficient tumours was different between both series: in the Brussels patients group, MMR deficient tumours were mainly located in the right colon (33/43 i.e. 77%) whereas in the Abidjan group they were predominant (10/11 i.e. 91%) in the left colon ($p<0.05$).

With regard to the involved proteins, 6/11 (55%) of the MMR deficient cases from Ivory Coast were characterised by loss of expression of MSH2 and MSH6 whereas this immunohistochemical staining pattern was observed in only 9/43 (20%) cases from Belgium.

Conclusion :

Our pilot study reveals marked differences in presentation of MMR deficient colorectal cancer between the two geographic regions. In contrast to Europe, MMR

deficient colorectal cancer in Ivory Coast is mainly found in male patients and in the left colon. Moreover, there are differences with regard to the involved mismatch repair proteins. Together with the younger age at presentation, these data suggest differences in epidemiology and biology of colorectal cancer in native Africans from West Africa compared to the European population.

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MULTIGENE SIGNATURES BASED RISK ESTIMATES IN ER+/HER2- BREASTS CANCERS: THE PREDICTIVE VALUE OF THE MAGEE EQUATIONS AND THE MEMORIAL SLOAN KETTERING SIMPLIFIED SCORE AND CHANGES IN ADJUVANT CHEMOTHERAPY USE.

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Abstract

Background:

Multigene signatures (MGS) may select women with estrogen receptor positive and human epidermal growth factor receptor 2 negative (ER+/HER2-) breast cancers where chemotherapy can be avoided. However, MGS are expensive and not reimbursed in Belgium. Several inexpensive statistical models based on multiple pathologic parameters have been developed to predict MGS results. We studied the predictive value of two such tools, in tumors with a low or high relapse risk based on MGS. We also recorded the change in the decision of the multidisciplinary meeting (MDM) to add chemotherapy, following MGS results.

Patients and Methods

We retrospectively included 103 primary operable HR+/HER-2- breast cancer patients (pN0-3a) diagnosed at the University Hospitals Leuven (UHL) from 2011 till now; all analyzed either by MammaPrint® (Agendia, Amsterdam; n= 8), OncotypeDX® (Genomic Health, Redwood City, n= 36) or Prosigna® (NanoString Technologies, Seattle, n= 59) because of uncertainty about the benefit of adjuvant chemotherapy during MDM. Based on UHL guidelines, patients are regularly stratified in clinically high (Clin-high) and low risk (Clin-low) groups during MDM. We calculated the Magee equations (ME) and Memorial Sloan Kettering simplified score (MSK) using hormone receptor status, HER-2 status, tumor size, nuclear score, Nottingham grade score and Ki-67. ME and MSK scores stratify patients into three risk categories (low, intermediate and high).

Results

Of 36 patients tested with OncotypeDX®, 17 (47%) were recurrence score (RS) low, 11 (31%) RS intermediate and 8 (22 %) RS high. Of 59 tumors evaluated by Prosigna®, 11 were risk of recurrence (ROR) low, 21 ROR intermediate and 27 ROR high. Of 8 patients tested with P 25

MammaPrint® half were low and half were high risk. All MSK or ME-high cases were classified by MGS either as high risk (respectively n=12, n=5) or intermediate risk (n=1, both) without MGS-low-risk. However, all MammaPrint® tested tumors resulted in ME-intermediate score. Table 1 shows how the predictive value of MSK and ME-scores. In total, we observed a chemotherapy switch according to the MGS results in 46/103 (45%) patients. The MGM considered 61/103 (59%) patients to be Clin-high and thus eligible for chemotherapy, but only 33 (54%) of them received chemotherapy after MGS results. Of the 42 (41%) patients that were Clin-low, adjuvant chemotherapy was given to 18 (43%) patients after MGS results. Eventually, adjuvant chemotherapy was given to 51 patients following MGS testing and resulted in 16% (n=10/61) relative and 10.5% absolute reduction.

Conclusion

The use of inexpensive statistical models based on multiple pathologic parameters can be helpful in selecting patients that may need MGS testing in case of uncertainty about adjuvant chemotherapy, and warrants further study. Integration of MGS results into MDM decisions, resulted in a substantial decisional switch and reduction in chemotherapy administration.

Table 1: Predictive value of MSK and ME in MGS tested tumors. Concordance between MSK/ME high or low scores in 71/103 patients with high (n=39) or low MGS risk (n=32) based on OncotypeDX® (ODx), MammaPrint® (MP) and Prosigna®

	MGS high-risk (n=39)			MGS low-risk (n=32)		
	ODx	MP	Prosigna	ODx	MP	Prosigna
MSK-high	7/8 (88%)	1/4 (25%)	4/27 (15%)	0/17 (0%)	0/4 (0%)	0/11 (0%)
ME-high	4/8 (50%)	0/4 (0%)	1/27 (4%)	0/17 (0%)	0/4 (0%)	0/11 (0%)
MSK-low	0/8 (0%)	1/4 (25%)	13/27 (48%)	10/17 (59%)	2/4 (50%)	6/11 (55%)
ME-low	0/8 (0%)	0/4 (0%)	2/27 (7%)	0/17 (0%)	0/4 (0%)	2/11 (18%)

Conclusion

The use of inexpensive statistical models based on multiple pathologic parameters can be helpful in selecting patients with ER+/HER-2- breast cancers that may need MGS testing in case of uncertainty about the addition of adjuvant chemotherapy after MDM, and warrants further study. Integration of MGS results into MDM decisions, resulted in a substantial decisional switch and reduction in chemotherapy administration.

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CASE REPORT

ASSOCIATION OF AN OVARIAN ADULT GRANULOSA CELL TUMOUR AND ENDOMETRIOID INTRAEPITHELIAL NEOPLASIA : A CASE REPORT AND COMPREHENSIVE REVIEW OF LITERATURE

Q. Fontanges, K. El Ali, J.C. Noël / ULB/Erasme Hospital, Brussels

Content:

An 83-year-old woman with persistent vaginal bleeding was addressed to gynaecology practice. Hysteroscopic endometrial curettage was performed and pathological examination of the specimen morphological features was strongly suggestive of endometrial intraepithelial neoplasia (EIN).

Further examination included pelvic ultrasonography and revealed a left 27mm solid adnexal mass showing cystic and haemorrhagic areas, regular margins and ultrasonographic features of benignity.

The patient underwent total hysterectomy with bilateral salpingo-oophorectomy and pathological examination of the left ovarian tumour demonstrated an adult granulosa cell tumour (AGCT). Molecular biology profile, using Next Generation Sequencing technology including a panel of 16 genes involved in gynaecological tumours, found a somatic point mutation in FOXL2 gene (402 C>G) pathognomonic for AGCT.

Adult Granulosa cell tumour belong to the pure sex cord tumours category of the 2014 WHO Classification and are by far one of the most frequent sex cord-stromal tumours, although still accounting for only 1% of all ovarian tumours. Despite its low malignant potential and somehow indolent behaviour, overall recurrence rate reaches 20% to 30% and remains unpredictable, staging being the only reliable prognostic factor to this day.

Adult Granulosa cell tumour is one of the most common ovarian tumours exhibiting estrogenic manifestation such as post-menopausal bleeding. Indeed, tumour cell exhibit morphological, biochemical and hormonal features of normal proliferating pre-ovulatory granulosa cells including expression of follicle-stimulating hormone receptor and inhibin.

Its association with endometrial proliferative disorders is well documented ranging from 21.5% to 71% for endometrial hyperplasia and from 1.3% to 13.2% for endometrial cancer.

Association between AGCT and EIN should be known and methodical assessment of endometrial mucosa performed when AGCT is diagnosed. Nevertheless latest data suggest that systematic hysterectomy might not be justified in the treatment of AGCT if patients have no endometrial abnormalities at diagnosis.

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A SYSTEMATIC REVIEW ABOUT THE POSITIVE PREDICTIVE VALUE OF HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION ON CYTOLOGY FOR THE HISTOLOGICAL DIAGNOSIS OF CERVICAL INTRAEPITHELIAL NEOPLASIA 2 OR MORE.

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Introduction:

As cervical cancer is a major health problem, regular cervical screening to make an early diagnosis can help prevent cervical cancer, through identifying and treating pre-invasive cervical lesions.

Aim :

The aim of this review is to evaluate the worldwide correlation between the cytological screening and histological outcome in the diagnosis of cervical cancer, more specifically the correlation between HSIL on cytology and histological CIN2+. Learning if cytology brings up information about the probability to discover a high grade cervical intraepithelial neoplasia, would imply that the cytological screening program is a valuable tool on its own.

Methods :

An electronic search was carried out in Medline (through Pubmed) and Cochrane (last searched in November 2016), supplemented with the related article feature in Pubmed and snowballing. Article selection (predefined in- and exclusion criteria), data extraction and methodological quality assessment (QUADAS) were evaluated by two independent reviewers.

Results :

After identifying 1065 articles, 24 articles were included in this systematic review, representing 51.962 cytological HSIL women in total. The mean CIN2+ percentage in cytological HSIL women is 65,1% (range: 45,4% – 95,2%). The mean CIN3+ percentage in cytological HSIL women is 43,9% (range: 36,4% – 62,1%).

Conclusion :

In this systematic review, the mean CIN2+ percentage in cytological HSIL women is 65,1%. This implies that the correlation between HSIL on cytology and histological CIN2+ is demonstrable but not strong enough to only rely on cytology for the screening and the therapeutic process.

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CASE REPORT

A RARE SYNCHRONOUS B-CELL AND T-CELL PTLD IN THE LIVER: A CHALLENGING DIFFERENTIAL DIAGNOSIS WITH REJECTION.

A. Camboni, I. Ferreira, M. Komuta / Cliniques universitaires Saint Luc, Woluwe Saint Lambert

Introduction:

Post-transplant lymphoproliferative disorders (PTLDs) is a complication after solid organ transplantation. The majority of PTLDs are of B-cell origin and are associated with Epstein–Barr virus (EBV) infection. T-cell PTLDs are significantly rarer and infrequently associated with EBV. We report a rare case of concomitant B- and T-cell PTLD in an child after liver transplantation and discuss the histopathological differential diagnosis in liver biopsy.

Case report:

An Algerian 3 year old girl liver transplant recipient presented fever and viral symptoms 15 months after liver transplantation. Clinical examination revealed a cervical mass as well a splenomegaly. Elevated liver's markers, an hypergammaglobulinaemia, as well as a positive EBV-PCR were reported. Clinically a PTLD associated to liver rejection was suspected.

Biopsy from cervical mass revealed an EBER positive early PTLD with plasmacellular hyperplasia. A biopsy from liver was taken at the same time, which revealed portal tract and sinusoidal dilatation infiltrated by monoclonal T-lymphocytes. There was no evidence of endothelialitis or biliary damage. A diagnosis of monomorphic T-cell PTLD manifested as hepatosplenic T-cell lymphoma (HSTCL) with a concomitant EBV-positive B-cell PTLD was performed. A treatment by anti-CD20 was started.

In view of aggravating patient's symptoms, a new liver's biopsy as well as bone marrow and splenic biopsies were performed. All the biopsies revealed a sinusoidal infiltration of monoclonal EBER-/CD3+/CD8+/TCR betaF1-/TCR M1+/- lymphocytes confirming the diagnosis of monomorphic T-cell PTLD manifested as HSTCL.

Discussion:

HSTL is a rare and aggressive extranodal lymphoma derived mostly from cytotoxic γδ T-cells. Up to 20% of HSTL arise in the setting of chronic immune suppression, most commonly kidney transplantation, and to a lesser extent heart, liver, hematopoetic, lung and multivisceral transplant.

The most common feature present in almost all patients with HSTL is infiltration by malignant T lymphocytes of the spleen and the liver. However, EBER- T-cell PTLD may show considerable overlap with liver rejection, and differentiation may be difficult in allograft biopsy specimens, as many activated CD8+ T cells infiltrate the transplant at the time of rejection. Moreover a T clonal expansion may be observed during rejection making the

differential diagnosis with T-cell PTLD more challenging. Furthermore, coexistence of PTLD and rejection may occur and can be diagnosed by the presence of a polymorphic inflammatory portal infiltrate, images of endothelialitis and damage to bile duct epithelium. The morphologic assessment, a comprehensive immunophenotyping and a molecular analysis are critical to the correct classification of these complex lesions.

To our knowledge this is the first case of concomitant B-cell PTLD and HSTL in a pediatric liver transplant recipient.

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NOTES

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Model used for illustrative purposes only.

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