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Dear colleagues

The current times conspire against the full exercise of Medicine, including clinical and diagnostic aspects. The present and future difficulties, of recognized dimensions, present us with a challenge.

Effective treatment is founded on accurate diagnosis, the reason and priority of our work.

The determination that animates us is expressed more eloquently in the following words of Fernando Pessoa:

“Agir, eis a inteligência verdadeira. Serei o que quiser. Mas tenho que querer o que for. O êxito está em ter êxito, e não em ter condições de êxito. Condições de palácio tem qualquer terra larga, mas onde estará o palácio se não o fizerem ali?”

Welcome to the first Updating Course on Anatomic Pathology, Centro Hospitalar do Porto, 2012!

José Ramón Vizcaíno
**UPDAtING COURSE ON ANATOMIC PATHOLOGY**  
**CENTRO HOSPITALAR DO PORTO 2012**

**Organized by**  
Centro Hospitalar do Porto  
Serviço de Anatomia Patológica  

**October 13-16, 2012 – Porto, Portugal**

**Course Description:** We are pleased to announce the 1st edition of our course entitled “Curso de Actualização em Anatomia Patológica Centro Hospitalar do Porto 2012”. The course is organized by Centro Hospitalar do Porto, Porto [Portugal] and shall provide the highest level of subspecialty instruction by a distinguished faculty recognized in the fields of Anatomic Pathology. The educational event shall include 3 days of anatomic pathology theoretical and practical sessions related to breast, salivary gland, lymphomas, bone and soft tissues, cutaneous melanocytic lesions and pleuropulmonary and mediastinal areas, highlighting diagnostic challenges, illustrating potential pitfalls and how to avoid them, and highlighting the role of complementary molecular techniques and their application in patient care. The surgical pathology blocks will be anticipated by corresponding clinical satellite conferences, giving emphasis on National contributions.

The greatest educational feedback is expected for community hospital physicians, in particular, pathologists and residents. The course goal is to provide participants with practical and meaningful information relevant to diagnostic task, prognostic assessment and therapy guidance of cancer patients. The course is intended for a broad audience; however, it is primarily designed for pathologists devoting most of their efforts to practical surgical pathology. Course organizers encourage the participation of anatomic pathology, general and thoracic surgery, dermatology, ENT and oncology residents.

**Location of the Course:** The course will be held in Porto, the second largest city of Portugal, in the west coast. It can be reached by airplane through Porto International Airport where various flights are available – for more information http://www.ana.pt/en-US/Aeroportos/porto/Porto/Pages/Homepage-Porto.aspx

**Course Venue:** Centro Hospitalar do Porto, Largo Prof. Abel Salazar, 4099-001 Porto.

**Official language:** The surgical pathology course will be taught in English. Simultaneous translation services will not be available. The clinical satellite conferences will be lectured in English or Portuguese.

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**SCIENTIFIC COMMITTEE**

Giovanni Falconieri, M.D.  
João Castro e Melo, M.D., Ph.D.  
Margarida Lima, M.D., Ph.D.  
Rui Henrique, M.D., Ph.D.  
José Ramón Vizcaíno, M.D.  
Pedro Farrajota, M.D. (scientific course coordinator)

**ORGANIZING COMMITTEE**

André Coelho, M.D.  
Diane Esteves, M.D.  
Francisca Costa, M.D.  
José Garcia, M.D.  
Renata Dias, M.D.  
Rita Sampaio, M.D.  
Manuela Moreira (secretariat)  
Marta Aguiar (secretariat)  
Pedro Farrajota, M.D.  
José Ramón Vizcaíno, M.D. (main organizer)
MEETING PROGRAMME

SATURDAY, OCTOBER 13th, 2012

Registration
Registration desk will open at 16.00

18:00 – 18:15 Course introduction and welcome to the participants
F. Sollari Alillegro, M.D., J.R. Vizcaíno, M.D., R. Henrique, M.D., Ph.D.

18:15 – 19:00 Inaugural Lecture
Updates on the clinical diagnosis and treatment of breast cancer
F. Marques, M.D., Ph.D.

SUNDAY, OCTOBER 14th

09:00 – 10:30 Breast theoretical session:
1. Updates on core needle biopsy of the breast
2. Issues in the assessment of axillary sentinel lymph nodes
Nour Sneige, M.D., Ph.D.

10:30 – 11:15 Sysmex conference:
How do use TNM in breast cancer when axillary dissection is not performed?
New tools to predict nodal involvement.
Vicente Peg, M.D.

11:15 – 11:45 COFFEE BREAK & SLIDE READINGS

11:45 – 12:45 Breast practical session:
Nour Sneige, M.D., Ph.D.

Clinical Satellite Conference*
11:45 – 12:45 Updates on the clinical diagnosis and treatment of salivary gland tumors
António Moreira da Costa, M.D.

12:45 - 14.00 LUNCH

14:00 – 15:30 Salivary gland theoretical session:
1. Molecular Advances in Salivary Gland Pathology and their Practical Application
2. Salivary duct carcinoma - update
Alena Skálová, M.D., Ph.D.

15:30 – 16:30 COFFEE BREAK & SLIDE READINGS

16:30 – 17:30 Salivary gland practical session
Alena Skálová, M.D., Ph.D.

Clinical Satellite Conference*
16:30 – 17:30 Flow cytometry in the diagnosis of lymphomas
– the experience of Centro Hospitalar do Porto
Margarida Lima, M.D., Ph.D.

MONDAY, OCTOBER 15th

09:00 – 10:30 Lymphoma theoretical session:
1. Follicular lymphoma microenvironment
2. The pathology of aggressive non-Hodgkin’s lymphomas
Pedro Farinha, M.D., Ph.D.
10:30 – 11:30 **COFFEE BREAK & SLIDE READINGS**

11:30 – 12:30 **Lymphoma practical session**  
*Pedro Farinha, M.D., Ph.D.*

**Clinical Satellite Conference***

11:30 – 12:30 **Updates on the clinical and radiological diagnosis of bone and soft tissue tumors**  
*João Pires, M.D. & Pedro Cardoso, M.D.*

12:30 – 14.00 LUNCH

14:00 – 15:30 **Bone and soft tissues theoretical session:**  
*Molecular and cytogenetic aspects of soft tissue and bone tumors*  
*Eduardo Zambrano, M.D., M.S.*

15:30 – 16.30 **COFFEE BREAK & SLIDE READINGS**

16:30 – 17:30 **Bone and soft tissues practical session**  
*Eduardo Zambrano, M.D., M.S.*

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**TUESDAY, OCTOBER 16th**

09:00 – 10:30 **Theoretical session on cutaneous melanocytic lesions**  
1. **Melanocytic lesions at special sites**  
   *Boštjan Luzar, M.D., Ph.D.*

2. **Benign melanocytic lesions looking malignant**  
   *Boštjan Luzar, M.D., Ph.D.*

10:30 – 11:30 **COFFEE BREAK & SLIDE READINGS**

11:30 – 12:30 **Practical session on cutaneous melanocytic lesions**  
*Boštjan Luzar, M.D., Ph.D.*

**Clinical Satellite Conference***

11:30 – 12:30 **Updates on pathology of pulmonary hypertension**  
*Abílio Reis, M.D.*

12:30 – 14.00 LUNCH

14:00 – 15:30 **Theoretical session on pleuropulmonary and mediastinal diseases**  
1. **Overview of pleural mesothelioma**  
   *Giovanni Falconieri, M.D.*

2. **Thymic carcinoma: update of current**  
   *Giovanni Falconieri, M.D.*

15:30 – 16.30 **COFFEE BREAK & SLIDE READINGS**

16:30 – 17:30 **Practical session on pleuropulmonary and mediastinal diseases**  
*Giovanni Falconieri, M.D.*

**Clinical Satellite Conference***

16:30 – 17:30 **Ipilimumab: immunotherapy on the treatment of advanced melanoma**  
*Ana Raimundo, M.D.*

**Closing lecture**

17:30 – 18:00 **The Portuguese Cancer League new strategies against cancer**  
*Vítor Veloso, M.D.*

**Closing remarks**  
*J.R. Vizcaino, M.D. & R.Henrique, M.D., Ph.D.*
COURSE FACULTY

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Serviço de Radiologia
Centro Hospitalar do Porto

Margarida Lima, M.D., Ph.D.
Serviço de Hematologia Clínica
Centro Hospitalar do Porto

Pedro Cardoso, M.D.
Serviço de Ortopedia
Centro Hospitalar do Porto

INAUGURAL LECTURE

Franklin Marques, M.D., Ph.D.
Serviço de Oncologia
Centro Hospitalar do Porto

CLOSING LECTURE

Vítor Veloso, M.D.
Presidente do Núcleo Regional do Norte da Liga Portuguesa Contra o Cancro.
AKNOWLEDGMENTS

To the Conselho de Administração do Centro Hospitalar do Porto (C.H.P. Board), for the immediate reception of this initiative
To the Departamento de Ensino, Formação e Investigação (DEFI) (Department of Teaching, Education and Research) for their unconditional support
To the Course Faculty for their knowledge and generosity
To the Participants for their determination and support
To the Sponsors for sharing a common interest in fighting disease and cancer
To all those we cannot name

The Course has been made possible through the kind support of

ACTELION  AXA  redefinimos / standards

Bristol-Myers Squibb  LIGA PORTUGUESA CONTRA O CANCRO

Pfizer  Roche  Sysmex

Leica  Olympus

Samsung  Smart TV  Real Common Vision
Franklim Marques, M.D., Ph.D. is Senior Consultant of Medical Oncology (1998) and Head of the Oncology Division, Department of Medicine, Hospital de Santo António, Centro Hospitalar do Porto, Portugal since 2002. He has been exclusively dedicated to Medical Oncology (1989), became member of The European Society for Medical Oncology (ESMO) in 1990 and has received the title of European Oncologist by ESMO in 1991.

Professor Franklim Marques was Invited Associated Professor of Medicine, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Porto, Portugal from 1986 to 1997 (Disciplina de Clínica Médica III) and Invited Assistant Professor of ICBAS, responsible for Oncology Area (Disciplina de Clínica Médica II) since 1998. He has been mentor in the Master Degree thesis of 24 ICBAS medical students.
Updates on the clinical diagnosis and treatment of breast cancer

Cancers or neoplasms are autonomous growths of tissues that have escaped normal restraints on cell proliferation and exhibit varying degrees of fidelity to their precursors. Based on the GLOBOCAN 2008 estimates, about 12.7 million cancer and 7.6 million cancer deaths are estimated to have occurred in 2008; of these, 56% of cases and 64% of deaths occurred in the economically developing word.

Global burden of cancer continues to increase largely because of the aging and growth of the population alongside an increasing adoption of cancer–causing behaviors. More than 50% of cancers could be prevented implementing what is already known, mostly through lifestyle modifications and higher–cost interventions.

Worldwide, breast cancer is the most frequently diagnosed life–threatening cancer in women and the leading cause of cancer death among women. In 2008, the estimated numbers of breast cancer cases and deaths in Europe were 425147 and 128737 respectively, accounting for 28.2% the total new cancer cases and 17% of the total cancer deaths.

Increased public awareness and improved screening have led to earlier diagnosis, at stages amenable to complete surgical resection and curative therapies. Consequently, survival rates for breast cancer have improved significantly.

The general approach to evaluation of breast cancer has become formalized as triple assessment: clinical history and examination, imaging (usually mammography and/or ultrasonography), and needle biopsy. Surgery is considered primary treatment for early breast cancer. Some patients with early–stage breast cancer are cured with surgery alone. Because breast cancer has the tendency to early metastasize, adjuvant treatment (loco regional radiotherapy, hormone therapy, chemotherapy and directed therapy) have indication in the great majority to treat micrometastatic disease, or breast cancer cells that have escaped the breast but which have not yet had an established identifiable metastasis. Updates on axillary lymph node dissection, sentinel lymph node dissection strategies and adjuvant therapy are presented and discussed.

The pathogenesis of breast cancer is still poorly understood, but epidemiologic, molecular and genetic studies are outlining complex risk factors. Breast cancers exhibit diversity in histopathology, molecular features, and overall patient outcomes. Tailored treatment recommendations are summarized.

Notes
Nour Sneige, M.D., Ph.D. is Professor at the Department of Pathology, Division of Pathology/Lab Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas since 1993. She graduated in Science (1967) and Medicine (1972) in Damascus University, Damascus, Syria and has received extensive postgraduation training in the United States. Following the pioneer work of cytopathologists at Karolinska Hospital, and under the directorship of her former chairman Dr. John Batsakis, lead the establishment of the Fine Needle Aspiration Clinic at MD Anderson Cancer Center in 1985. This clinic was the first of its kind in the medical center and was also one of a handful of clinics operated by cytopathologists in the nation. This service along with the FNA service of deep-seated lesions, which is performed by the radiologists and whose specimens are immediately assessed by the cytopathologists, grew rapidly and has replaced open surgical biopsy in many cases. As the Director of Cytopathology for the past 10 years, she was instrumental in creating and maintaining the Section of Cytopathology as a premier center of excellence for the practice of “the state of the art” cytopathology. As early as 1989, she introduced the Breast Pathology Subspecialty Service, standardizing specimen evaluation, pathology reporting and assessment of prognostic and predictive markers. She established the Breast Tissue Bank and was the pathologist in charge for that specialty for 10 years. She also initiated in–house testing of breast cancer cases for HER–2 gene amplification by FISH technique, which proved to be the most reliable technique for targeted monoclonal antibodies therapy. In addition to serving the MDACC patients, this lab has been sought out by outside investigators for their research as well as validation studies. Her contribution to clinical research is demonstrated throughout the numerous publications in high quality peer reviewed journals. Her commitment to five editorial advisory boards and presentations of several invited lectures at the national and international levels also attest her accomplishment and reputation in the field of breast pathology and cytopathology. She is frequently sought after as a consultant on difficult breast cases, inside and outside the institution.
Update on Core Needle Biopsy of Non-palpable Breast Lesions

Nour Sneige, M.D.
UT MD Anderson Cancer Center
Houston, Tx

CNB vs Surgical Excision
- Same accuracy (1-2% delayed false neg)
- Less invasive, less expensive
- Spares most patients (benign) unnecessary surgery
- Empowers patient to decide on treatment options

CNB of Breast Lesions
1. Technical considerations
2. Diagnostic problems
3. Controversies: to excise or not to excise

Imaging Guidance Systems
- Ultrasound
- Stereotactic mammography
- Magnetic resonance (MRI)

Ultrasound Guidance System
System of choice (lesion imaged with US)
- Less expensive
- More readily available
- No radiation exposure
- Lesions not amenable to stereo (tail of axilla, close to chest wall)
- Less time consuming

Stereotactic Guidance System
- Can be used for wider range of lesions: masses and calcifications
- Breast and lesion are immobilized accurate sampling ± 2 mm

MRI
Demonstrates cancers not detected on mammography or sonography
- Sensitivity 100%
- Specificity 37-97%

Needle Biopsy Devices
- Automated (spring loaded gun)
- Directional Vacuum Assisted (DVAB)
**Advantages:**

**DVAB vs Automated**
- Faster (1.4 min. vs. 17 min.)
- Twice the weight (34 vs. 17 mg)
- Tissue shift by bleeding, avoided
- Contiguous sampling

**Advantages:**

**DVAB vs Automated**
Underestimation of ADH
- 41% Automated needle
- 15% DVAB (Mammotome)
Cales retrieval 99-100%


**CNB Size**
Gauge: 18, 16, 14, 11, 8-g
Cales: 11-g V more accurate than 14-g V/A

**Optimal Numbers of CNB**
Solid masses: 4-5 cores
Calcifications: > 10 cores

**Specimen Radiography - CNB**
- Confirm Cales
- Cales must correspond to those on mammography

**Segregation of Cores with and without Cales on Specimen Radiographs**
Diagnostic yield of malignancy (DVAB):
- Cores with cales: 84%
- Cores without cales: 71%
Same diagnosis: atypical/malignant in 76%
Equally careful attention should be given to cores with no cales

Margolin et al. Radiology 2004; 233: 251-254

**Radiologic-Pathologic Correlation**
- Pathologist should review specimen radiograph (cales)
- Pathologist should be provided with:
  1) Specimen radiograph
  2) BI-RADS category
Imaging-Histologic Discordance

0.9 - 6% of cases
Discordance is an indication for surgical excision (24% malignant)

Diagnostic Problems- CNB

- Tubular lesions (tubular CA vs. adenosis)
- Papillary lesions (pap CA vs. papilloma)
- Mucinous lesions (mucocle vs. mucic CA)
- Fibroepithelial lesions (FA vs. PT)
- In situ vs invasive
- Ductal vs lobular carcinoma in situ

Diagnostic Problems- CNB

1. Benign or malignant?
2. In situ or invasive?
3. In situ lobular or ductal?

Breast Biopsy Claims from 1998-2003

<table>
<thead>
<tr>
<th>Claims</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>42</td>
<td>15.5%</td>
</tr>
<tr>
<td>False Negative</td>
<td>20</td>
<td>48%</td>
</tr>
<tr>
<td>False Positive</td>
<td>22</td>
<td>52%</td>
</tr>
</tbody>
</table>

21% of all breast bx claims involved CNB


Diagnostic Errors in Stereotactic/ Palpable CNB of Breast

1. Misdiagnosis of DCIS, SA and adenosis as invasive ductal carcinoma.
2. Misdiagnosis of LCIS as low-grade DCIS.
3. Failure to recognize small, easily over-looked foci of invasive lobular carcinoma.


Indeterminate Lesions at CNB

- Ancillary studies
- If in doubt, second opinion / defer to surgical excision

Controversies: To Excise or Not to Excise?

- Lobular Neoplasia
- Papilloma
- Mucocle
- Radial Scar
- Flat epithelial atypia
- ADH

Consensus Meeting on Image Detected Breast Cancer – The American College of Surgeons 2005

Patients with high-risk lesions, including ADH, ALH and LCIS found on percutaneous biopsy may have DCIS or invasive cancer at the same site and should generally undergo surgical excision. This incidence of missing such important findings is markedly reduced with the use of vacuum assisted biopsy and large gauge needles.
**Consensus Meeting on Image Detected Breast Cancer – The American College of Surgeons**

For some individuals with high-risk histologic findings, in whom careful correlation of imaging and histologic findings is concordant, or breast MRI is normal, follow-up without surgical excision may be reasonable.

**LN in CNB is Associated With a Low Risk of DCIS/IC**

- 92 LCIS/ALH on CNB
  - 7 cancers on excision
    - 3 (3%) in area of bx site (1 DCIS, 2 IDC)*
    - 2 away from bx site
    - 2 after negative bx site excision

*One interpretive error (ADH on core)

Renshaw et al. AJCP 2006; 126: 310-313

**LCIS/ALH on CNB - MDACC**

Multidisciplinary approach

No excision required: if

1. Cales only
2. Completely removed by DVAB
3. Classic LCIS

**Papillary lesions on CNB: to Excise or Not to Excise?**

- < 5% of breast biopsies
- Include benign atypical and malignant

**Reliability of CNB in the Diagnosis of Papilloma?**

Papilloma with no atypia
345 cases (15 series)

8 series: 0% upgrade to cancer
7 series: 2-20% upgrade to cancer

**Papillary lesions on CNB**

1063 subjects (16 series) with benign or atypical papillary lesions

138 (23%, R 6-39%) upgraded to carcinoma on excision


**Relationship of Mode of Biopsy of the Papillary Lesion With Malignancy at Surgical Excision**

<table>
<thead>
<tr>
<th>Mode of percutaneous biopsy</th>
<th>N</th>
<th>Malignant</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stereotactic</td>
<td>33</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>US- core</td>
<td>17</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td>US- FNA</td>
<td>30</td>
<td>9</td>
<td>30</td>
</tr>
</tbody>
</table>

**Relationship of Pathologic Characteristics of Papillary Lesions With Malignancy at Surgical Excision**

<table>
<thead>
<tr>
<th>Pathologic Diagnosis bx</th>
<th>Ca/cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papilloma/papillomatosis</td>
<td>6/36</td>
<td>17</td>
</tr>
<tr>
<td>Atypical papillomatosis/ papilloma with ADH</td>
<td>2/7</td>
<td>29</td>
</tr>
<tr>
<td>“Pure” papillary lesion</td>
<td>9/28</td>
<td>32</td>
</tr>
<tr>
<td>Papillary lesion with atypia</td>
<td>2/9</td>
<td>22</td>
</tr>
</tbody>
</table>

Department of surgery and radiology.

**Lesions Yielding a Benign, Concordant Diagnosis of Papilloma at Percutaneous Biopsy May Warrant Surgical Excision**

35 papillomas on CNB
25 excised, 10 mammo F/U > 2 yrs
Excision: 5 Cancers (14%)

Liberman, et al. 2006 AJR 186: 1328-1334

**Lesions Yielding Benign Papilloma at Percutaneous Biopsy and Cancer at Surgery**

<table>
<thead>
<tr>
<th>Type</th>
<th>Size (mm)</th>
<th>Guidance/ Needle</th>
<th># of Cases</th>
<th>Target</th>
<th>Interval (mm)</th>
<th>Surgical Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Caes</td>
<td>1.2</td>
<td>Sf / II V</td>
<td>59</td>
<td>Excised</td>
<td>1</td>
<td>DCIS seen from bx side</td>
</tr>
<tr>
<td>2 Mass</td>
<td>1.2</td>
<td>Sf/14A</td>
<td>4</td>
<td>Sampled</td>
<td>7</td>
<td>IDC and DCIS at periphery of pap lesion</td>
</tr>
<tr>
<td>3 Mass</td>
<td>0.8</td>
<td>US/14A</td>
<td>5</td>
<td>Sampled</td>
<td>25</td>
<td>DCIS at periphery of papillaoma</td>
</tr>
<tr>
<td>4 Mass</td>
<td>0.6</td>
<td>US/14A</td>
<td>4</td>
<td>Sampled</td>
<td>21</td>
<td>DCIS in papillaoma</td>
</tr>
<tr>
<td>5 Mass</td>
<td>0.8</td>
<td>Sf/ II V</td>
<td>14</td>
<td>Excised</td>
<td>22</td>
<td>IDC, gr 3 and DCIS, 1.8cm, adjacent with pap</td>
</tr>
</tbody>
</table>

**Lesions Diagnosed As Papilloma on CNB - MDACC**

No excision:
- Small (up to 1.5 cm)
- DVAB samples
- Concordant imaging and histologic findings (size)

**Mucinous Lesions**

- < 1% of CNB specimens
- Range: Mucocele-like to mucinous carcinoma

**Mucocele-like Tumors- CNB**

Among 20 cases of MLL with no atypia, no carcinoma was found on excision.

CNB is highly reliable for accurate Dx of mucinous lesions

Wang et al AJCP 2007
Cardor et al Histopath 2004
Roeschard AJCP 2007

**Radial Scars**

- Stellate
- Radiating central fibroelastic stroma
- Nonprolif epith.

**Results of Surgical Excision of Radial Scars Without Atypia Diagnosed on CNB**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Biopsy Technique</th>
<th># cases</th>
<th>DCIS/SC at Excision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bershaw</td>
<td>14 A</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Lee</td>
<td>14 A</td>
<td>4</td>
<td>3 DCIS</td>
</tr>
<tr>
<td>Jackman</td>
<td>14 A</td>
<td>5</td>
<td>2 DCIS, 1 DCIS</td>
</tr>
<tr>
<td>Philpotts</td>
<td>14 A; II M</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Kirwan</td>
<td>14 A</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Cowan</td>
<td>14 A</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>3 (4%)</td>
<td></td>
</tr>
</tbody>
</table>

17
Radial Scar on CNB

Brenner et al. AJR 2002; 179:1179-1184

198 lesions (11 institutions)
157 lesions
(102 excised, rest f/u 24 m)

CA at excision:
RS with ADH on CNB 28%
RS no ADH 4%

Radial Scar on CNB

Brenner et al. AJR 2002; 179:1179-1184

Dx of RS on CNB is likely to be reliable when:
- No associated ADH
- >12 cores obtained
- VAD used

ADH at CNB: MDACC Approach

ADH limited to ≤2 foci (DVAB) had no worse lesion on excision, provided that most of calc are removed

Ely et al., AJSP 2001; 25, 1017-1021
Streige et al., AJCP 2003;119:248-253

ADH in DVAB of Microcalcifications

Nguyen, Albarracin, Whitman, Lopez, Sneige

140 patients (86.4% excision)
ADH associated with calc in the absence of a mass can be categorized into 2 different risk groups.

ADH in DVAB of Microcalcifications

Nguyen, Albarracin, Whitman, Lopez, Sneige

1. ADH associated with significant atypia and/or necrosis are most likely to be associated with carcinoma and should excised

Nguyen, Albarracin, Whitman, Lopez, Sneige

2. ADH without these features, regardless of extent of involvement, and with >95% removal of the targeted calc, is associated with a minimal risk (<3%) of carcinoma and may undergo mammographic f/u only

Nguyen, Albarracin, Whitman, Lopez, Sneige

Notes

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Issues in Assessment of Axillary Sentinel Lymph Nodes

Nour Sneige, M.D.

MD Anderson Cancer Center
Houston, Texas

Axillary Lymph Nodes Status

Most significant prognostic factor

Goals of ALN dissection:
Accurate staging and guiding treatment selection
---potential complications---

Sentinel Lymph Node (SLN)

First node to receive lymphatic drainage from the area of the primary tumor

Lymphatic mapping with SLN biopsy for breast cancer introduced in 1990

Advantages

• Accurate (negative SLNs accurately predicts absence of metastasis in the remaining axillary nodes (95% to 100%)
• Safe (significant reduction in surgical morbidity)
• More sensitive to detect small metastases than ALN dissection – small number of LNs removed can be subjected to a more detailed pathologic evaluation.—standard of care---

Sentinel Lymph Node - Objectives -

• Definition of micrometastases
• Prognostic Significance
• Identification/Intraoperative evaluation (H&E, IHC and mol.)
• Conclusions

Minimal Disease in ALN

• IHC and SLN —— detection of minimal disease in ALNs
• 6th AJCC 2002: lower limit for micromet >0.2 - 2.0 mm
• Isolated tumor cell clusters (ITCs) (up to 0.2 mm)

ITCs vs Micrometastasis

7th AJCC 2010

ITCs: Small clusters of cells not greater than 0.2 mm in largest dimension ---or as nonconfluent or nearly confluent tumor cells not exceeding 200 cells in a single histologic LN cross section

ITCs

• ITCs may be detected by routine histology or by IHC methods.
• Regional LNs are designated as pN0(i+) or pN0(i+)sn, as appropriate and number of ITC involved nodes should be noted (regardless of # of nodes involved).
• Cells in different LN cross or longitudinal sections or levels of the block are not added together.
**Micrometastases**  
Tumor deposits greater than 0.2 mm but not larger than 2 mm in largest dimension or > 200 tumor cells identified as single dispersed or as a nearly confluent in a single slide (even if <0.2 mm).

PN1mi (sn), as appropriate, and the number of involved nodes should be noted.

---

**Size of a Tumor Deposit**  
Largest dimension of any group of cells that are touching one another (confluent or contiguous tumor cells) regardless of whether the deposit is confined to the LN, extends outside the node (extranodal or extracapsular extension), or is totally present outside the LN and invading adipose tissue.

---

**Desmoplastic stromal reaction present:**  
the combined contiguous dimension of tumor cells and fibrosis determines size of the metastases.

---

**Prognostic Significance of Micrometastases in Axillary Lymph Nodes**  
In studies that have evaluated complete axillary dissection:

- Occult mets not associated with poor prognosis  
  Khan et al. 2006  
  Chen et al. 2007

- Associated with poorer outcome than N0:  
  Sakorafas et al. 2004  
  Mabenco et al. 2006  
  Kuijt et al. 2005 (if no adjuvant chemo given)

---

**Prognostic Significance of Micrometastases and ITCs in Axillary Nodes**

**SLNs**

A. Incidence of further metastases in non-sentinel axillary lymph nodes

B. Survival differences

---

**Prognostic Significance of Micrometastases and ITCs in SLNs**  
Incidence of further metastases in NSLNs (#1228 pts):

<table>
<thead>
<tr>
<th>SLN</th>
<th>NSLN mets</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.5%</td>
<td>15%</td>
</tr>
<tr>
<td>26%</td>
<td>21%</td>
</tr>
</tbody>
</table>

SLN stratified as to size of micromets 1mm vs 1-2 mm: ITCs and micromets up to 1 mm had a significantly lower risk of additional mets (Viale et al.)

---

**Prognostic Significance of Micrometastases and ITCs in SLN**  
Survival differences: Controversial  
Associated with a significantly shorter disease-free interval than was SLN negativity (Reed et al.2009, de Boer et al.2009, Truong et.al 2010)

Not significant (majority adjuvant)  
Hansen et al. 2009; Weaver et al. 2011 (78%); Giuliano et al. (Z0010, 83%)

---

**Effect of Occult Metastases on Survival in Node-Negative Breast Cancer**  
NSABP-B32 occult mets (neg SLN at initial exam but pos. at 2 widely spaced additional H&E levels and (HC) were detected in 15.9% of 3887 patients

Occult metastases were an independent prognostic variable (with respect to overall survival p=0.03, DFS p=0.02, and D- D-FS p=0.04), however the magnitude of the difference in outcome at 5 years was small 1.2 percentage points (5 yr estimates of OS among pts were 94.6% and 95.8% respectively).

Weaver et al, N Engl J Med 2011
Sentinel Lymph Node
- Objectives -
  - Definition of micrometastases
  - Prognostic Significance
  - Identification/Intraoperative evaluation (H&E, IHC and mol.)
  - Conclusions

Pathologic Evaluation of SLNs

Goals:
Identify all macrometastases (larger than 2 mm, pN1) and most micrometastases (pN1mi)

Gross Handling of LNs

Serial sections at 1-to-2 mm intervals through and parallel to the longest axis. Entire LN should be submitted for evaluation. Each LN is submitted in a separate cassette or identified by colored ink to permit accurate assessment of the total number of LN and number of involved nodes. -- short half-life and limited penetration of technetium, health risks to those handling SLNs are negligible (ASCO Guidelines 2005).

Intraoperative Examination of the Sentinel Node

Allows immediate axillary dissection when metastasis is found in the SLN.

For every 100 patients who have SLNs evaluation intraoperatively, 16 to 17 will have positive nodes and 8 to 9 will have false-negative results.

Each institution must have a policy on intraoperative assessment or deferral to permanent sections.

Intraoperative Examination of the Sentinel Node

• Standard techniques:
  - Imprint cytology or evaluation of cells scraped from the cut surface of the node
  - Frozen section (carry the risk of significant destruction of potentially diagnostic tissue).
  - Suspicious findings should be reported as not diagnostic for tumor and deferred to paraffin section
• Molecular techniques

Comparison of FS and TIC

• FS more sensitive than TIC (approximately 80% to 90% versus 70% to 80%).

• Both techniques commonly fail to detect micrometastases and ITCs.

• Rapid CK-IHC may reduce IO false negative cases, but require additional time, personnel and cost.

Potential Sources of Trouble

1. Nevus Cell aggregates
2. Benign glandular inclusions
3. Displacement of benign epithelium
4. Extramedullary hematopoiesis
5. Sinus histiocytosis
6. IHC CK positive dendritic cells
**Sampling SLNs/Paraffin Section Levels—Current Practice**

No consensus among pathologists regarding how many H&E and CK IHC levels should be examined.

**CAP:** single microscopic section from each LN block, no IHC.

**ADASP:** “several” microscopic sections from each block and also do not routinely recommend IHC.

**Limited step sections:** (top level plus 1 or 2 sections cut at 200- to 500-micrometer intervals into the block).

**Comprehensive analysis:** The European Institute of Oncology group performs FS analysis of the entire sentinel node in multiple step sections separated by 50-um intervals.

---

**Observations from NSABP B-32**

1. No mets larger than 1 mm identified by comprehensive protocol.

2. Patients classified as neg for occult mets: 8.9% chance of undetected ITCs and 2.2% chance of missed micromets.

3. ITCs detected: 22% chance that ITCs are misclassified micrometastasis.


---

**Sampling SLNs/Paraffin Section Levels and Use of IHC**

**NSBPP-32** clinical trial conclusion:

Observed difference in 5 yrs survival 1.2 percentage points between patients in whom occult mets were detected and patients in whom occult mets were not detected was not significant.

Raised the argument against analysis of additional tissue levels or routine IHC-CK for SLN evaluation.

---

**Correlation between Histopathologic Examination of the SLN and BLN Assay Results**

<table>
<thead>
<tr>
<th>Histology</th>
<th>BLN Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Metastases &gt;2 mm</td>
<td>51</td>
</tr>
<tr>
<td>Micrometastases 0.2-2 mm</td>
<td>5</td>
</tr>
<tr>
<td>ITC</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
</tr>
</tbody>
</table>


---

**Observations from NSABP B-32**

1. More mets are detected when more levels are examined.

2. Size of undetected mets are related to the thickness of unexamined nodal tissue.


---

**Our practice**

- Serial sections of LN at 1-2 mm
- 1 H&E and 3 unstained
  (if H&E negative, stain one IHC-CK)

To facilitate Dx of small-volume mets.

Detection of each possible ITC should not be the goal of routine histologic evaluation.

---

**Issues/concerns**

Pathologists: No consensus on methods of SLN evaluation.

ASCO members: Not following guidelines.
Recommendations:
- Update of ASCO guidelines
- Communications with oncologists to identify cases requiring further analysis.

**Adjuvant chemotherapy (78% in B-22 vs 85% in Z0011)**

Yes ↓

One H&E
NO levels or IHC

Adjuvant assessment tools for prognosis and prediction of benefit include:
- Adjuvant Online
- St. Gallen consensus
- Oncotype DX or MamaPrint

Micromets: sole indication for admin of chemo in only 2.1% of cases (24051 patients in Denmark study, *Eur J Cancer* 2011)

Identification of occult metastases may be limited to remaining cases (intermediate risk for recurrence). Use of 2 wide spacing cut/IHC is appropriate.

Notes
Challenges and diagnostic clues in breast pathology

Nour Sneige, M.D.

MD Anderson Cancer Center
Houston, Texas

CASE 1

A 62 year old woman underwent a core needle biopsy for microcalcification followed by a segmental mastectomy.

Differential Diagnosis:

Ductal Carcinoma In Situ (solid growth) vs Pleomorphic Lobular Carcinoma In Situ
Pleomorphic Invasive Lobular Carcinoma

Page et al. Diagnostic Histopathology of the Breast 1987
Weinder and Semple (1992) 16 cases
Eusebi (1992) 10 cases
Clayton (1998) 12 cases
Middleton (2000) 39 cases

In situ counterpart (45%) was always in association with invasive ca.
Pathologic Features of LCIS

Classic: Foote and Stewart, 1941
(Lobulocentric, solid prolif. small monotonous cells--does not produce a clinically identifiable lesion)

LCIS (not ductal): loss of E-cadherin exp. & differentially express HMWks (34bE12)
most Morphologically ambiguous in situ lesions ductal or lobular types

Variants of LCIS (Non-classic type)

- Sapino et al. 2000 "Mammo detected LCIS" (all 10 had calcs with necrosis, all E-cad neg, intermediate size nuclei, 1 pleo, entire breast in 3, 4 with ILC)
- Shin and Rosen 2002 (21 cases, abstract) "Florid LCIS with necrosis(calcifications)" 1-2 NG, LOH e-cad
- Sneige et al 2002 PLCIS (NG 2-3, x4 lymph, high Ki-67, nec/calcs) (10/24)
- Fadare et al 2006 classic LIN/LCIS with necrosis (small to intermediate), 6/18 pure in situ. Invasive mostly lobular

Variants of LCIS- Common features

- Mammographic features (calcs) and central necrosis associated with HG DCIS
- Nuclear grade: low to intermediate (Florid/LCIS with necrosis) to high (PLCIS)
- Higher proliferation index >10%
- Concurrent invasive carcinoma 25%
LCIS Variants in Breast CNB Potential for Misdiagnosis and Upgrade at Surgical excision

- Among 75 cases of solid DCIS, 10 (13.3%) were reclassified as LCIS (5 PLCIS, 4 LCIS with necrosis, 1 classic). One-tenth of “solid DCIS” dx on CNB in the past may represent LCIS variants.
- 7 (25%) upgraded to invasive in surgical excision.

Sullivan et al. Arch Pathol Lab Med 1024, 2010

What is your diagnosis?

- Patient was referred with a diagnosis of low grade DCIS on core needle biopsy.
MRI: a second lesion in a different clock position noted.

MRI guided bx was performed.
Final pathology

2 separate foci:
PLCIS involving sclerosing adenosis
Invasive pleomorphic lobular carcinoma,
4 mm in extent

E-cadherin Expression in LCIS

- Complete loss common
- Reduced expression
- Fragmented, patchy or incomplete pattern of membrane staining (some E-cad +)
- other markers (HMW-CK, B-catenin, p120-catenin cytoplasmic but membranous in DCIS) Uncertain: In situ ca with mixed DL/indet.

E-cadherin protein expression in ILC

- A transmembrane protein that mediates cell-to-cell cell adhesion as well as several signal transduction pathways
- For its function, E-cad must be associated with catenins (α, β, and γ-catenins and p120)
- 16% of ILC were E-cad positive ----but abnormal expression of one or more of the catenins complex (diffuse cytoplasmic expression of catenins, in particular p120)
- Expression of E-cad in ILC should not preclude its diagnosis

Fisher: Ductulolobular carcinoma in situ (NSABP B-17)
Page and Anderson: In situ carcinoma of both lobular and ductal type
Rosen text book (1997) are best regarded as “examples of ductal and lobular carcinoma in situ”

PLCIS - Molecular genetics:
Gain of 1q and loss of 16q (features typical of LCs, but are not seen in high grade ductal lesions).
In addition, genetic changes more analogous to high grade DCIS, gains of c-myc, Her-2, gains on 8p+q and 13q and losses on 1p, 8p, 12p, 14q, 18q and 19+q.

Because of the putative ductal element of DLCIS, we have tentatively regarded post-operative local breast irradiation as appropriate for its treatment. This prompted its exclusion from this study”.
NSABP – Protocol B-17

PLCIS
Natural History
??

Treatment and Follow-up
All 7 alive (4 - 32 m, means 17 m)
1 recurrent CA at 12 months after lumpectomy (initial lesion <1 mm from margin)
Fadare et al. 1 treated with XRT recurred after 7 years, recurrent LCIS with necrosis?

Clinical Implications of Margin Involvement by PLCIS
26 pts (Fu 4-108 months, mean 46)
23% chemoprevention, 15% XRT, 23% both.
LCIS at M (23%), 1 mm (27%), 1.1-2 mm (15%), >2 mm (36%).
Recurrent PLCIS: 1 patient with a positive margin (overall rec regardless of treatment 3.8%). --similar to that of low- or intermediate-grade DCIS.
Conclusion: 2 mm negative margins is appropriate

Management of LCIS variants:
as low/intermediate DCIS with free resection margins

Notes
CASE 2

A 45 year old woman presented with a palpable, well defined breast mass that was diagnosed as fibroadenoma on FNA. However, over a follow-up period of 3 years, the mass increased in size from 2.7 cm to 4 cm. An ultrasound-guided core biopsy was performed followed by excision.
**Diagnosis:**

Phyllodes tumor, histologically benign

---

**Distribution of ducts and stroma in PT VS FA**

PT: lack of uniformity in the distribution of stroma and ducts and stromal heterogeneity, with some areas showing expanded stroma, a leaf-like architecture, and increased cellularity.

FA: uniform distribution throughout the lesion

---

**Features Used in the Evaluation of the Malignant Potential of a Phyllodes Tumor**

1. Infiltration
2. Mitotic count > 4 MFs/10 HPF
3. Extensive stromal overgrowth
   - defined as > one x4 field without glandular structures (now modified to x2).
4. Significant stromal pleomorphism and atypia

From Kempson RL. (Based on Hawkins et al Cancer 1992, 69:141-47)

---

**Features Used in the Evaluation of the Malignant Potential of a Phyllodes Tumor - continued**

All four present: malignant

Stromal overgrowth with one or more of the other three: malignant

One or more of the other three present without stromal overgrowth: uncertain

Severe nuclear atypia + mitoses >10/10 HPF: malignant

None of the four: benign

---

Prominent intracanicular pattern

PT

Stromal heterogeneity, expansion and inc. cellularity
World Health Organization Classification

<table>
<thead>
<tr>
<th></th>
<th>Benign</th>
<th>Borderline</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stromal hypercellularity</td>
<td>modest</td>
<td>modest</td>
<td>marked</td>
</tr>
<tr>
<td>Cellular pleomorphism</td>
<td>mild</td>
<td>moderate</td>
<td>marked</td>
</tr>
<tr>
<td>Mitosis</td>
<td>few if any</td>
<td>intermediate</td>
<td>numerous (more than 10 per 10 HPF)</td>
</tr>
<tr>
<td>Margins</td>
<td>well circumscribed, pushing</td>
<td>intermediate</td>
<td>invasive</td>
</tr>
<tr>
<td>Stromal pattern</td>
<td>uniform stromal distribution</td>
<td>heterogeneous stromal expansion</td>
<td>marked stromal overgrowth</td>
</tr>
<tr>
<td>Heterologous stromal differentiation</td>
<td>rare</td>
<td>rare</td>
<td>not uncommon</td>
</tr>
<tr>
<td>Overall average distribution (IMP)</td>
<td>&lt;10%</td>
<td>10%</td>
<td>30%</td>
</tr>
</tbody>
</table>

PT - Rosen’s Breast Pathology

<table>
<thead>
<tr>
<th></th>
<th>Benign</th>
<th>LMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stromal</td>
<td>uniform but can be heterogeneous</td>
<td>Heterogenous</td>
</tr>
<tr>
<td>Borders</td>
<td>usually well defined but invasive</td>
<td>Invasive</td>
</tr>
<tr>
<td>Mitosis</td>
<td>1-2/HPF</td>
<td>2.5/HPF</td>
</tr>
<tr>
<td>Cellularity</td>
<td>moderate to marked</td>
<td>Moderate resembling fibromatosis/LG F5</td>
</tr>
<tr>
<td>Pleomorphism</td>
<td>slight to moderate</td>
<td></td>
</tr>
</tbody>
</table>

Features Used in the Evaluation of the Malignant Potential of a Phyllodes Tumor

1. Infiltration
2. Mitotic count > 4 MFs/10 HPF
3. Extensive stromal overgrowth
   (defined as absence of epithelial component in at least one x4 field (now modified to x2).)
4. Significant stromal pleomorphism and atypia

From Kempson RL (Based on Hawkins et al Cancer 1992, 69:141-47)

Features Used in the Evaluation of the Malignant Potential of a Phyllodes Tumor - continued

- All four present: malignant
- Stromal overgrowth with one or more of the other three: malignant
- One or more of the other three present without stromal overgrowth: uncertain malignant potential
- Severe nuclear atypia + mitoses >10/10 HPF: malignant
- None of the four: benign

PT, Uncertain Malignant Potential/LMP

Circumscribed, Mod. cellularity but mitoses >4/10HPF
### Prognosis

- **Benign PT:** less risk for local recurrence (20%) and will not metastasize. Interval to rec. longer. Initial recurrences are histologically benign in almost all instances.
- **Low grade PT:** more likely than a benign PT to recur locally (>20%), earlier interval to local recurrences, recurrences are likely to be histologically high grade more often. Probability (<5%) of metastasis.
- **High grade PT:** also susceptible to local recurrence. Mets occur in about 25%.

### PT, LMP

Infiltrative borders with many adjacent nodules (pericellular stromal proliferation). Margins were also positive. Re-excision margins free.

Recurred 7 yrs later as pleomorphic liposarcoma.

### Correct Dx: PT, UMP

36 y/o woman, recurrent “FA” x 3, at 1-2 yrs intervals infiltrative tumor border with intrapped fat, 2nd nodule adjacent to main lesion with hypercellularity, resection margin involved.

### Recurrent Sarcoma (pleomorphic liposarcoma), 7 yrs later

### Clinicopathologic Features and Long-Term Outcomes of 293 PTs of the Breast

#### Local Recurrences
- 14.4%, equal frequency between benign and malignant PTs
- LR in Benign and LMP: half transformed to malignant

#### Distant recurrences
- 2% of all patients
- 6% of malignant PT

---

*Barrio et al. Annals of Surgical Oncology. 14(8):7963-7970*
Clinicopathologic Features and Long-Term Outcomes of 293 PTs of the Breast

Death due to PT
- Rare (5 / 293) patients
- All 5 had tumors labeled as malignant
- All 5 shared uniformly poor histologic features: large size ≥7 cm, infiltrative borders, stromal overgrowth, marked stromal cellularity, ≥5 mitoses/10HPF and necrosis

Immunochemistry in PT
- Stromal cells variably CD 34+
- No consensus on the usefulness of p53 and Ki-67 as risk predictors of PT
- C-kit is expressed in few cases
- CD10 (FA rare), in small proportion of benign PT, 20% UMP, and 50% of MPT—not useful in dx work-up--

CNB VS FNA in the Preoperative Diagnosis of Fibroepithelial Lesions
- A review of 6 published studies, total 201 patients (Jacobs et al)
  - CNB is preferred diagnostic modality
  - FNA: high false-negative rate (25%)
  - CNB: Distinction between FA and PT may not be possible in all instances (fibroepithelial lesion, favor--)

Morphologic features favoring PT in CNB
- Mitotic activities (increased Ki-67)
- Increased cellularity
- Stromal overgrowth
- Infiltrative edges, and entrapped fat
- Tissue fragmentation (fragments of stroma lined by epithelium on one or two opposing edges)
- Large size >3 cm and rapid tumor growth
  - even features in core c/w FA
CASE 3

A 66 year old woman
1.5 cm. breast mass.
Core needle biopsy followed by excision.
Referred with a diagnosis of “invasive ductal carcinoma”
Assessment of invasion (in situ vs invasive carcinoma)

- Basement membrane components
- Myoepithelial cell markers

Basement membrane components

Type IV collagen and laminin:
Continous BM around benign epithelium and in situ ca
Patchy or absent around invasive ca

Markers are not entirely reliable

Myoepithelial cell markers

An intact layer of myoepi surrounding the epithelium is the hallmark of benign breast tissue and in situ proliferation

Most commonly used:
- Smooth muscle actin (SMA)
- Smooth muscle myosin heavy chain (SMMHC)
- Calponin
- p63

<table>
<thead>
<tr>
<th>Markers</th>
<th>Localization (staining pattern)</th>
<th>Sensitivity</th>
<th>Myofibroblasts</th>
<th>Vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>Cytoplasmic (small arches boling towards luminal cells)</td>
<td>Most sensitive</td>
<td>Strong</td>
<td>Yes</td>
</tr>
<tr>
<td>Calponin</td>
<td>Similar to SMA</td>
<td>Less than SMA</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>SMMHC</td>
<td></td>
<td>Less than SMA and calponin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p63</td>
<td>Nuclear (dotted line)</td>
<td>Slightly less sensitive than SMA</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

P63 may stain epithelial cells
Myoepithelial cell markers

Caveats:

- MECs may be attenuated in distended benign ducts and in large ducts of in situ carcinoma.
- Sensitivity of some MEC markers is lower/absent in DCIS-associated MECs and benign sclerosing than in normal MECs.
Myoepithelial cell markers

Caveats:

- IHC + = noninvasive
- Lack of immunoreactivity should not be considered conclusive evidence of stromal invasion.

Diminished Number or Complete Loss of Myoepithelial Cells Associated with Metaplastic and Neoplastic Apocrine Lesions

Apocrine proliferation with sparse nuclear p63 compared with the normal gland on the right

The same ducts showing a continuous layer of cytokeratin positivity with calponin


Diminished Number or Complete Loss of Myoepithelial Cells Associated with Metaplastic and Neoplastic Apocrine Lesions

DCIS immunostained for SMA (A) and SMMHC (B)


DCIS-associated myoepithelial cells

- Kalof et al: 25 cases, decreased expression of CD10 (82%) and SMMHC (52%). Complete absence of CD10 (32%), and SMMHC (13%)
- Zhang et al: 2 cases of DCIS complete absence for any of 8 MEC markers
- Yeh and Meis: 1 case SMMHC less intense than that of calponin or p63

Decrease to absent expression of one or more MEC marker when compared with normal MECs

Phenotypic Alterations in Myoepithelial Cells Associated with Benign Sclerosing Lesions of the Breast

48 BSL were evaluated using 7 myoepithelial markers.

- MEC associated with BSL showed reduced expression of CK5/6, in 11.5% of cases, SMMHC in 20.9%, CD10 in 15%, p63 in 9% and calponin in 6.6%.
- In 15.9% of cases, complete absence of staining for CK 5/6. None of the cases showed reduced expression for SMA or p63.

The proportion of radial scars/complex sclerosing lesions and sclerosing adenosis with reduced expression was significantly different for CD10 (26.3% vs 9%), and p63 (17.4% and 0%).

Conclusions: myoepithelial cells associated with BSL of the breast may show immunohistochemical differences from normal myoepithelial cells.

Phenotypic Alterations in DCIS-associated Myoepithelial Cells

100 cases of DCIS (81 without invasion and 19 with associated invasive cancer) were evaluated for 9 MEC markers: SMA, SMMHC, calponin, p63, GATA3, CD10, and p53.

DCIS associated MECs showed **decreased expression** (when compared with normal MECs): 76.5% for SMMHC, 34% for CD10, 30% for CK5/6, 17% for calponin, 12.6% for p63, 4% for p75, and 1% for SMA.

Reduced MEC expression of SMMHC more frequent in HG than in non-HG DCIS (84.8% vs 51.5%) and completely absent in 54.7%.

Benign Sclerosing lesion on CNB, negative for SMA/p63

Sclerosing lesion, favor benign/indeterminate

Recommend excision for a definitive Dx

Phenotypic Alterations in Myoepithelial cells

MEC associated with DCIS, BSL, apocrine and hyperplastic lesions may show decreased expression when compared with normal MECs: 76.5% for SMMHC, 34% for CD10, 30% for CK5/6, 17% for calponin, 12.6% for p63, 4% for p75, and 1% for SMA.

Loss of myoepithelium is variable in solid papillary carcinoma (SPC) of the breast

Intracystic or Solid papillary Papillary Carcinomas of the Breast

Myoepithelial markers absent:

Invasion Indeterminant

Comment: Available outcome data indicate that they seem to have an excellent prognosis with adequate local therapy alone.
CASE 4

A 74 year old woman presented with a 1.3 cm. mass in the left breast. She had a previous history of FNA x2. Excisional biopsy was performed.
### Spindle Cell Lesions

- Monophasic lesions
  - Spindle cells:
    - Bland
    - Pleomorphic

- Biphasic lesions
  - Epithelial component:
    - Benign
    - Malignant

### Monophasic Bland-Looking Spindle Cell Lesions of the Breast
- Fibromatosis like spindle cell carcinoma
- Almost all positive for at least one CK
- Reactive spindle cell nodules after CNB
- Nodular fasciitis
- Inflammatory myofibroblastic tumors
- Myofibroblastoma
- Fibromatosis
- Pseudoangiomatous hyperplasia (PASH)

### Reactive Spindle Cell Nodule of the Breast After CNB/FNA
- 18 cases after CNB/FNA (interval 6-38 days, av. 16.4 days)
- Associated with pap or CS lesions (15 cases)
- Non encapsulated 1.5-9 mm
- Interlacing fascicles of plump spindle cells, small blood vessels, inflammatory cells
- Negative for AE1/AE3 and HMW keratin but expressed SMA

### Myofibroblastoma
- Most often men > 40 yr
- Sharply circumscribed
- Fascicles of spindle cells
- Bands of hyaline collagen

Inflammatory Myofibroblastic Tumor

- Rare
- Well defined
- Clonal

Fibromatosis of the Breast

- Rare, locally aggressive
- Women 13-80 yrs (mostly child bearing)
- Solitary firm suspicious mass
- Poorly demarcated, 0.5-10 cm (av. 2.5 cm)

Variations:

- Infiltrative margins
- Prominent epithelioid component
- Mono or multinucleated giant cells with atypia and myxoid hyaline change

Immunoprofile: Vimentin, desmin, & SMA and variably for CD34

Monophasic Bland-Looking Spindle Cell Lesions of the Breast

- Fibromatosis-like spindle cell carcinoma
- Reactive spindle cell nodules after CNB
- Nodular Fasciitis
- Inflammatory myofibroblastic tumors
- Myofibroblastoma
- Fibromatosis
- Pseudoangiomatous hyperplasia (PASH)
Monophasic Bland-Looking Spindle Cell Lesions of the Breast

- Fibromatosis-like spindle cell carcinoma
- Reactive spindle cell nodules after CNB
- Nodular Fasciitis
- Inflammatory myofibroblastic tumors
- Myofibroblastoma
- Fibromatosis
- Pseudoangiomatous hyperplasia (PASH)

PASH

- Incidental microscopic findings (23% of bx), often associated with a benign or malignant condition
- Diffuse involvement or localized palpable or nonpalpable mass (0.4% of bx)
- Gynecomastia (25% of cases)

Symptomatic: Firm, non-tender mass/ FA Rec, 1-15 cm

Fibromatosis of the Breast - Immunoprofile

- CK & S100-p
- β catenin
- ER, PR, AR and pS2 neg

PASH

- Slit like spaces lined by myofibroblasts separated by bands of hyalinized tissue
- No atypia, no mitotic activity
- CD34+, Vim+, SMA+ (CD31 and vascular markers neg)

CASE 4

Diagnosis:
Low Grade (Fibromatosis Like) Spindle Cell Carcinoma of the Breast
Monophasic Bland-Looking Spindle Cell Lesions of the Breast

- Fibromatosis-like spindle cell carcinoma
- Almost all positive for at least one CK
- Reactive spindle cell nodules after CNB
- Nodular fasciitis
- Inflammatory myofibroblastic tumors
- Myofibroblastoma
- Fibromatosis
- Pseudoangiomatous hyperplasia (PASH)

Low Grade (Fibromatosis Like) Spindle Cell Carcinoma of the Breast

- A variant of metaplastic carcinoma (fibromatosis-like tumor; distinct resemblance to fibromatosis and similar propensity to local recurrence)
- Women, mean age 64 yrs (40-85 yrs)
- Tumor size 1-7 cm

IHC in Spindle Cell Carcinoma of the Breast

- Wide spectrum polyclonal anti CK antibody or enhanced CK immunostain
- Sensitivity AE1/AE3 improved with antigen retrieval but 20% of tumors still immunonegative (Adem et al. 2002)
- Pankeratin (MNF 116) most sensitive (93%), CK 14 (90%), AE1/AE3 (41%) (Carter et al. 2006)

Myoepithelial Differentiation in Metaplastic Ca of the Breast

- Frequent expression of basal and myoepithelial keratins: 34BE12, CK5 and CK14 (Dune et al.)
- Frequent pos: SMA, CK14, S100-p, p63, maspin and P-cadherin (Rats-Filho)
- P63 in 10/10 (Koker and kleer)
- CD10 (80%), p63 (70%), SMA (50%), S100 (45%) (Lebl et al.)
- SMA, p63 and CK 14 (39%) Carter et al.

IHC equally support that at least some are Spindle squamous carcinoma

---the only morphologically distinctive feature that distinguishes MEC is its clear-cut emanation from the myoepithelial cell layer, as this has never been described in spindle cell squamous cell carcinomas or other types of metaplastic carcinomas of the breast. Both MEC and spindle squamous carcinoma are derived from the myoepithelial cell layer or they have a common stem cell.

Myoepithelial Carcinoma of the Breast: a clinicopathological and immunohistochemical study of 15 diagnostically challenging cases

Bazza N, Zekry N, Charpin C, Tavassoli FA

Virchows Arch (2011) 457:337-345

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Spindle cell squamous carcinoma are even more difficult to distinguish from MEC based on morphology, and the immunoprofile of the two tumor types also overlaps (both tumors are positive for high molecular weight CKs and p63).
**Biologic Behavior**

- Gobbi et al: 18/30 with F/U, 6-88 mon (m. 27m)
  - 8 local rec

- Sneige et al: 16/24 with F/U, 8-90 mon (m. 33m)
  - 2 local rec (5 & 32 m)
  - 2 distant mets (lung)

- Carter et al: (6 LG) 2 died of disease (21 & 12)
  - 2 alive with mets
  - No axillary nodal metastasis

**FLSCC of the breast with 2+ cellularity.** The patient developed lung metastasis 2 years after the initial diagnosis.

**Metastatic Spindle Cell Tumors- Melanoma**

- HMB-45 and Melan A can be neg
- Strong S100-protein would support melanoma over carcinoma

**Spindle Cell Lesion of the Breast- Summary-**

- A battery of cyokeratins including CK14,17 and 34BE21
- FLSCC have potential for local recurrence and distant metastasis
- FLSCC have a decreased rate of nodal involvement
- A subset of FLSCC show myoepithelial differentiation

**Metastatic Spindle Cell Melanoma**

- 25 yrs old woman with axillary mass misdiagnosed as metaplastic breast carcinoma
Spindle Cell Sarcoma (Low/High Grade)

- Very rare (dx by exclusion)
- Leiomyosarcoma: strong diffuse reactivity for SMA+ and Actin
- Fibrosarcoma: herringbone growth
- Follicular dendritic cell tumors: CD 35 & CD 21 pos.
- Periductal stromal tumor/phylloides

Immunoprofile of Various Spindle Cell Tumors of the Breast

<table>
<thead>
<tr>
<th>Tumor</th>
<th>SMA</th>
<th>S-100</th>
<th>Pax 8</th>
<th>Act 8</th>
<th>CD 35</th>
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<th>ITIH3</th>
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Notes
Vicente Peg Cámara graduated in Medicine and Surgery in the University of Zaragoza and received the Pathology Residency Training at the University Hospital Vall d’Hebron, Barcelona and also did an observational stage at the Massachusetts General Hospital in Boston (USA). Then he returned to the Department of Pathology, H.U. Vall d’Hebron joining the Staff Attendings and becoming the group leader of Breast Pathology. Since then he has led several research projects related to breast cancer and has participated in several national and international clinical trials. He is the author of numerous publications and usual participant and speaker at scientific meetings. His main research focuses on cell signaling pathways in breast cancer and its relation to neoadjuvant chemotherapy treatments as well as hypoxia states and in the study of breast sentinel lymph node. He is an Associate Professor at the Universitat Autonoma de Barcelona since 2012.
How do use TNM in breast cancer when axillary dissection is not performed? New tools to predict nodal involvement.

Since its first description and despite the passage of years, the study of the sentinel lymph node (SLN) has always had the problems of evaluation subjectivity and difficult analysis standardization. The advent of molecular techniques such as the One Step Nucleic Acid Amplification (OSNA) from Sysmex (Kobe, Japan) has managed to make it an automated, standardized and reproducible technique. However, even with the efforts to achieve a SLN unified analysis, many studies have shown that between 40% and 60% of cases (analyzed by conventional techniques), the SLN is the only affected node, being the remaining axillary lymph node dissection (ALND) unnecessary. Furthermore, the results of the recently published study Z0011 from A.E. Giuliano group have shown that even having 1 or 2 positive SLNs, the ALND can be avoided in a particular subgroup of patients.

One of the direct consequences of the ALND removal is the preclusion to make a correct axillary lymph node staging (pN). In turn, there remains a non-negligible group of patients who meet the inclusion criteria for the A.E. Giuliano study which are directly pushed into axillary surgery, regardless of the size of the metastases in the SLN. Finally, we must not forget that this study includes primarily a subgroup of patients with good prognosis. For these and several other reasons, nomograms that predict the axillary status taking into consideration a series of clinicopathological parameters are still being used today for therapeutic decisions, being the most well-known the one proposed by the Memorial Sloan-Kettering Cancer Center, New York.

The OSNA method provides a new element in the SLN study, the tumor load, defined as the number of copies/μL of CK19 mRNA. Little is known of this value so far, so we decided to study its possible predictive value for axillary status. For this we have defined the concept of Total Tumor Burden (Total Tumor Load – TTL) as the sum of the tumor burden of all affected SLNs of 108 patients (obtained from H.U. Vall d’Hebron de Barcelona and from H. Clínico de Salamanca) and elaborated a ROC curve. In the univariate analysis, the TTL was the best predictor of axillary involvement (p = 0.003), only influenced by the hormonal status. In multivariate analysis, the correlation persisted. The area under the curve was 0.715.

These data demonstrate that the TTL is a new available tool for the management of patients with breast cancer. Compared to other existing current nomograms, it is based on an objective, automated, reproducible and standardized variable that, in addition, can be obtained intraoperatively. Prospective studies are being carried out to know the real clinical significance of this variable. However, as of today, it seems useful as a predictor of axillary involvement and can help the individualized clinical management of certain patients with early breast cancer.

Notes
Graduated from the Faculty of Medicine, University of Porto, 1981
Resident of General Surgery in Hospital de Santo Antonio, 1985–91
Joined the Attending Staff of the Department of Surgery, Hospital Santo Antonio, 1992
Became Consultant Surgeon in 1999
Appointed to the Head and Neck, Breast and Endocrine Unit in 2008 (resigned in 2009 due to health condition)
Currently Assistant Head of the Department of Surgery, responsible for Post Graduate Education and Pre Graduate teaching
One hundred presentations in National and International Congresses and Symposia related to the Head and Neck and Endocrine Surgery
Ten scientific articles published in Portuguese medical journals related to the thyroid, parathyroid and Head and Neck Surgery
Member of the Portuguese Surgical Society and its Endocrine Section
Member of the International Surgical Society
Member of the Royal College of Surgeons of England
Updates in diagnosis and treatment of salivary gland tumours

I – Anatomy of the salivary glands

Salivary Glands are divided into major and minor.
The major salivary glands are paired and comprise the parotid, submandibular and sublingual glands.
The parotid glands are located in front of the ear, lying over the ascending ramus of the mandible, with an elongated shape and present a variable extension caudally, which is termed the tail of the parotid gland. They have a variable extension forward, toward the mouth, over the buccinator muscle and are drained by a short duct, Stensen’s Duct, which opens in the jugal mucosa opposite the second pre-molar tooth of the maxilla.
The parotid is not a lobulated gland, contrary to common description; rather, it is compact, with a superficial and a deep portion, and is traversed by the main and secondary branches of the facial nerve. This nerve is the most important anatomic relation of the parotid.
Other important structures that are intimately related to the parotid gland, and may have to be taken into consideration when operating on it are the external carotid artery and the retro-mandibular vein.
There is an important anatomical marker, which is very helpful in locating the main trunk of the facial nerve: it is called the pointer; the pointer is best assessed by pressing the tip of the index finger onto the space between the mastoid process and the cartilaginous portion of the external auditory canal: at the tip of the finger the stylloid process will be felt, and behind it will appear the facial nerve.
The submandibular gland is also paired. It is located on the anterior half of the submandibular triangle, bounded above by the horizontal ramus of the mandible, anteriorly by the midline and posteriorly by the anterior belly of the digastrics muscle.
It is elliptical and is drained by Wharton’s Duct, a short structure that opens at the floor of the mouth, near the fraenulum of the tongue. Its most important anatomic relations are the facial vessels, artery and vein, which traverse the gland, and the hypoglossal, lingual and marginal mandibular branch of the facial nerve.
The sublingual gland is a paired structure, located on either side of the lingual fraenulum, rod-like, and that has no single collecting duct, rather opening into the floor of the mouth by means of a variable number of orifices.
In addition, there are countless numbers of minor salivary glands, present in the mucosa of the mouth, labial, gingival, lingual, jugal and palatal. They drain directly into the mouth.

II – Physiology of the salivary glands

The main function of the Salivary Glands is the production of saliva.
Saliva is the watery fluid that keeps the mouth moist and performs a certain number of digestive and bactericidal functions.
Saliva is composed of water, variable amounts of mucous, sodium bicarbonate, and enzymes.
The larger the gland, the greatest the content in water and bicarbonate of its saliva, and the smaller the gland, the larger its production of mucus.
Saliva contains variable amounts of salivary amylase, which starts the break–down process of the simplest sugars contained in the diet. It also contains lysozime, similar to that contained in tears, which helps in controlling the bacterial flora of the mouth. Nonetheless, a human bite is the most infectious of all carnivore bites.
III – Benign salivary gland neoplasms

The salivary glands can harbour a variable number of benign neoplastic proliferations. The larger the gland, the likelier will a neoplasm be. The most common benign tumour that presents in the salivary glands is the Pleomorphic Adenoma. Other benign neoplasms include Whartin’s Tumour, Oncocytic Adenoma, Mio-epithelial Adenoma, various cysts and other less common types. Clinical presentation is dominated by the appearance of a lump or mass, which is characteristically painless, firm or soft in consistency, that exhibits a slow tendency to grow and that does not involve the facial nerve by direct extension. Diagnostic work-up is based on imaging studies (ultrasound or more seldom, CT scan or MRI) and FNA. Pre operative diagnosis can be established with a very high degree of accuracy. The treatment of choice, and almost the universal mode of treatment, is surgical excision of the affected gland. Surgery is variable, according to the affected gland, but, generally, involves the complete excision of the gland, with special attention to clear surgical margins, owing to the very high rate of local recurrence if resection is marginal, tangential or if diseased gland is intersected. If this occurs, consideration should be given to complementary treatment with Radiotherapy. Associated surgical morbidity is rare, with the main concern lying on nervous injuries, inadvertently incurred intraoperatively. Prognosis is excellent with cure rates approaching 100%.

IV – Salivary gland cancer

Salivary gland cancer is a very rare occurrence. It comprises only 0.5% of all malignancies and less than 5% of all head and neck cancers. As stated before, the larger the gland, the less probable will any tumour arising in it be malignant; conversely, most tumours of the minor and medium sized salivary glands will be malignant. These forms of cancer are characterized by a very diverse clinical course, which is dependent mainly on histopathological type, stage at diagnosis and initial effectiveness of treatment. The most common histological types are Mucoepidermoid Carcinoma, Squamous Cell Carcinoma, Acinic Cell Carcinoma, Adenoid Cystic Carcinoma and Adenocarcinoma NOS. They generally present as painless mass or lump, which exhibits a variable growth pattern. Signs of nervous involvement, such as facial or hypoglossal or mandibular marginal nerve palsy are indicative of an advanced stage. So is the presence of cervical lymphadenopathy. Diagnostic work-up is basically the same as for benign tumours: imaging studies (ultrasound, Quantitative Dynamic contrast-enhanced MRI, Diffusion- weighed MRI, CT scan, PET scan) and the use of FNA. A relatively new diagnostic approach consists in the use of US-guided Core biopsy. This last modality seems to be superior in diagnostic accuracy to FNA, but carries a higher risk of facial nerve injury. The mainstay of treatment for salivary Gland Cancer is Surgery, with performance of intra-operative frozen section examination whenever there is no certainty of pre-operative diagnosis. Surgical principles are the same also, only more stringent: radical gland excision, absolute clear margins. If there is pre operative confirmation of nerve involvement, nerves must be deliberately sacrificed. In the cases of suspected or confirmed pre-operative lymph node metastases, a Neck Dissection is mandatory: its variable forms – Classical Radical, Modified Radical, and Functional – will be determined by the extension of lymph node involvement, by tumour grade and by clinical staging of the neoplasm.
There are several available options for adjuvant therapy: Radiotherapy, Chemotherapy with conventional drugs and the newest available drugs such as TK, EGFR and HER 2 inhibitors. All these options offer very limited scope for success, and the best chance for cure lies in a properly performed radical operation. Surgical morbidity can be very distressing and disabling, especially where nerve lesions are concerned. This is most acute in those instances of facial nerve injury, be it accidental or deliberate. There are several forms of minimizing accidental injury, mainly by the use of intra-operative nerve monitoring with neuro stimulators.

If the facial nerve is sacrificed, either intentionally or inadvertently, there are several plastic surgery techniques, using nerve grafts, obtained from a variety of peripheral sensory nerves, such as the great auricular or sural nerves, muscle transfer flaps, such as the sternocleidomastoid flap, tarsorrhaphy, and so on.

For the treatment of Frey's Syndrome, or gustative sweating, which is owed to anomalous nerve regeneration, following the raising of facial skin flaps in the performance of parotidectomy, the most common approach use nowadays is Botox injection, subcutaneously, in the affected area. Prognosis is variable, and depends on histological type of cancer, its grade, pathological staging and completeness and effectiveness of therapy. Five year survival rates may vary between 75–100% for low grade acinic and polymorphous adenocarcinomas and 15–20% for undifferentiated, salivary duct and ex-pleomoromic carcinomas.
Alena Skálová, Ph.D., M.D., is Professor of Pathology at Medical Faculty in Plzen, Charles University Prague, Czech Republic. She graduated in Medicine in 1979, received her Board Certificates in Pathology 1st and 2nd degree in 1982 and 1989 respectively, presented her Ph.D. thesis in 1991, entitled “The role of myoepithelial cells in secretion and deposition of extracellular matrix in salivary gland tumors”, was Associate Professor of Pathology at the Medical Faculty of Charles University in Plzen (thesis entitled “Cell proliferation in salivary glands and in tumors of salivary gland origin) and Professor of Pathology since 2001.

She received Scholarship at the Department of Pathology of University of Helsinki 2 times (grants awarded by Finnish Ministry of Education (1991) and Yamagiwa-Yoshida Memorial International Cancer Study Grant – awarded by International Union Againts Cancer UICC in Geneva (1993)) and again a Study grant from UICC (International Union against Cancer) (1996). Her contribution to scientific research and medical literature is demonstrated throughout the numerous publications in high quality peer reviewed journals and as an author in several reference books including the W.H.O. Classification of Tumours. She has received numerous scientific awards and was committed to several invited lectures at the national and international levels, attesting her accomplishment and reputation in the field of Salivary Gland Pathology.
Molecular Advances in Salivary Gland Pathology and their Practical Application

The review describes the new findings in salivary gland pathology. Newly recognized entities include sclerosing polycystic adenosis (SPA) initially believed to be reactive/inflammatory lesion similar to fibrocystic disease of breast. Recent molecular study using the HUMARA (human androgen receptor assay) for clonality analysis demonstrated that SPA is clonal, and thus most likely neoplastic process (1). Three salivary gland tumors have newly described interesting molecular profiles that warrant discussion: mucoepidermoid carcinoma, adenoid cystic carcinoma, and a recently described tumor, mammary analogue secretory carcinoma (MASC).

Mucoepidermoid carcinoma (MEC) is a common salivary gland tumor that occurs in both major and minor salivary glands. The tumor has variable clinical course with some tumors having excellent behaviour while the others are characterized by rapid poor clinical outcome. Conventional clinicopathological parameters such as stage and grade are among the most significant prognosticators (2,3). However, histological grade is not entirely predictive of biological behaviour in all patients, particularly in low and intermediate grades. Therefore, a more objective consistent prognosticator is desirable for stratifying patients with MEC into appropriate treatment groups. A specific chromosomal translocation has been recently recognized in MEC, t(11;19)(q21;p13), which fuses MECT1 (mucoepidermoid carcinoma translocated-1) at 19p13 with MAML2 (mastermind-like gene family) at 11q21. The MECT1/MAML2 fusion transcript, present in more than half of MECs, is associated with lower histological grades and improved survival, suggesting both diagnostic and prognostic roles in clinical management (4). Moreover, MEC cases MECT1/MAML2 fusion positive form a favourable tumor subset that seems to be distinct from fusion-negative cases. When positive for the fusion, even “high-risk” patients including those with a higher histological grade or an advanced clinical stage showed an excellent prognosis (5).

Adenoid cystic carcinoma (AdCC) is a common tumor that occurs in both minor and major salivary glands. The tumor often has a relentless clinical course that includes late recurrences and distant metastatic disease. Recently, the translocation between chromosomes 6 and 9 was described both in AdCC of breast and salivary glands. This translocation fuses the MYB gene and the NFIB gene, which lead to characteristic chimeric transcript (6). The MYB/NFIB fusion transcript, present in at least one third of AdCCs, has emerged as a potential therapeutic target (7).

Mammary analogue secretory carcinoma (MASC) of salivary glands was recently described as a new entity characterized histologically by resemblance to secretory breast cancer (8). It was demonstrated that MASCs harbour a recurrent balanced chromosomal translocation t(12;15)(p13;q25), which leads to a fusion gene between the ETV6 gene from chromosome 12 and the NTRK3 gene from chromosome 15, exactly as in secretory breast cancer (8).

Another interesting new entity is cribriform adenocarcinoma of minor salivary gland origin affecting principally the tongue (CAT). This is an infiltrative tumor that occurs almost exclusively in the base of the tongue (9). Despite overlapping histological and immunohistochemical features with polymorphous low grade adenocarcinoma of minor salivary glands, CAT represents genuine entity with high frequency of cervical lymph node metastasis at presentation (9).

Finally, „dedifferentiation“ or high grade transformation of salivary carcinomas is discussed. The concept of „dedifferentiation“ in salivary gland tumor pathology means identification of clonal evolution of poorly differentiated (high grade) elements arising in low-grade carcinoma. This is in contrast with malignant transformation of originally benign tumors, such as carcinoma ex pleomorphic adenoma. In recent years, a variety of low grade salivary carcinomas, such as epithelial–myoepithelial carcinoma (10), adenoid cystic carcinoma (11), and acinic cell carcinoma (12), have been documented to progress to high grade carcinoma with consequent aggressive clinical behavior.
References


Salivary duct carcinoma: an update

Salivary duct carcinomas (SDC) are rare tumors with a very poor clinical outcome. SDC is defined in the 2005 World Health Organization (WHO) classification as „an aggressive adenocarcinoma which resembles high grade breast carcinoma” (1). These tumors comprise epithelial structures in solid, papillary–cystic, tubular, trabecular, and cribriform patterns and often display prominent nuclear and cellular polymorphism. Central comedo–like necrosis and perineural invasive growth are common findings. Most cases of SDC arise de novo, although some develop as the malignant component of carcinoma ex pleomorphic adenoma. The diagnostic clue in most cases of SDC is an intraductal in situ component comprising mostly solid and cribriform patterns similar to ductal carcinoma in situ (DCIS) of the breast.

Several morphological variants of SDC have been recently described. Mucin–rich variant is composed of areas of mucinous/colloid carcinoma in which clusters of carcinoma cells float in mucin pools (2). Invasive micropapillary variant of SDC is characterized by tumor cell clusters without fibrovascular cores, surrounded by a clear space, morphologically similar to micropapillary breast cancer. This variant appears to be associated with very aggressive clinical behaviour (3). Sarcomatoid variant of SDC comprises, in addition to conventional SDC, also admixed sarcomatoid component, within which there are anaplastic spindly and bizarre multinucleated giant cells (4). Although intraductal in situ SDC is not recognized as an entity by the 2005 WHO classification (1), occasional cases have been described (5) characterized by a pure intraductal proliferation of tumor cells, similar to intraductal carcinoma of the breast. In recent study, three cases of high grade in situ SDC were demonstrated, characterized by intraductal proliferation of neoplastic high grade ductal cells with co–expression of CK7, androgen receptors and HER–2/neu surrounded by complete myoepithelial cell layer (6).

Salivary duct carcinoma (SDC) and invasive ductal carcinoma (IDC) of the breast have well documented histomorphological similarities. Microarray studies have demonstrated that mammary IDCs can be subclassified into biologically and clinically distinctive molecular subgroups: luminal, HER–2 neu, basal–like, and normal breast–like cancers (7). Subsequently, a panel of immunohistochemical markers which can be used as a surrogate of gene expression analysis to classify breast carcinomas into molecular subgroups was described (8). The aim of our study was to apply an immunohistochemical panel previously validated for breast cancer to determine whether SDCs could likewise be classified into analogous molecular groups.
We used a modified version of the immunohistochemical panel for the classification of breast cancers into the molecular subgroups proposed by Nielsen et al. (8). Sixty four cases of salivary duct carcinomas (including SdC ex pleomorphic adenoma) were retrieved from the files and reviewed. All of them fulfilled the criteria for SDC according to the WHO classification (1). The tumors were stained with antibodies for estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), HER-2/neu, epidermal growth factor receptor (EGFR), cytokeratin 5, and cytokeratin 5/6. HER-2/neu status was assessed by immunohistochemistry and fluorescence in situ hybridisation (FISH). Cases were classified using criteria described previously (9). HER-2/neu phenotype was determined if the tumor cells expressed HER-2/neu protein (score 3+) and harboured the HER-2/neu gene amplification, regardless of the expression of other markers. Tumors lacking HER-2/neu expression which expressed ER, PR or AR, were considered of luminal phenotype, again regardless of the expression of other markers. Cases negative for HER-2/neu, ER, PR or AR which expressed either EGFR or CK 5/6 were considered of basal–like phenotype. Carcinomas which were negative for all markers were considered of indeterminate phenotype (9).

The majority of SDCs in our series represent the luminal subtype (23 cases, 38 percent of the invasive SDC). The second most prevalent group was HER-2/neu subtype (20 cases, 33 percent of the invasive SDC), whereas 12 cases fulfilled the criteria of the basal–like phenotype (19 percent of the invasive SDC). Nine cases of SdC (10 percent) were considered indeterminate phenotype.

Incidence of HER-2/neu positive subtype of SDC is consistent with other large recent studies (10, 11) but notably lower than in our previous report based only on 11 cases of SDC (12). The difference can be attributed to selection of cases using HER-2/neu immunostaining as a hallmark of SDC in our earlier study (12). In our new series, the cases were considered salivary duct carcinomas if they resembled a high grade ductal carcinoma of the breast, lacked any features suggestive of other specific types of salivary malignancy, and displayed expression of cytokeratin CK 7.

In conclusion, our results demonstrate that salivary duct carcinomas can be classified into molecular subgroups approximately equivalent to those in the breast. The study also shows that a considerable subset of SDC represents the HER-2/neu phenotype, and therefore selected patients with SDC may benefit from therapeutic use of trastuzumab (Herceptin) similar to the treatment in breast cancer.

References
CASE 1. A 50-year-old woman presented with painless swelling in buccal mucosa. The tumor had been present for approximately one year before surgery and it was gradually growing. The tumor was well circumscribed, and it measured 1 cm in diameter. No lymph node metastases were present. The patient is well alive with no recurrent disease within 6 year follow-up.

Diagnosis: Mammary analogue secretory carcinoma (MASC).

References
CASE 2. A slowly growing tumor of parotid gland in 17-y old girl measured 2.7x1.7x1.2 cm. Superficial parotidectomy was performed. The patient is well 5 years after surgery with no evidence of disease.

Diagnosis: Well differentiated acinic cell carcinoma with lymphoid stroma

References

CASE 3. 57-y-old man presented with long lasting painless swelling in right parotid gland. The CT scan showed a well circumscribed tumor 3 cm in diameter. The tumor was surgically removed by enucleation. No recurrence was observed in 4 year follow-up period.

Diagnosis: Sclerosing polycystic adenosis

References
CASE 4. 72-year old woman presented with cervical lymph node metastasis. The specimen was sent in consultation with preliminary diagnosis of primary pathologist was a lymph node metastasis of thyroid gland papillary carcinoma. In our consultation, we have suggested to examine the oral cavity, in particular the tongue of the patient. Two months later, the patient was operated, and surgical specimen disclosed a well circumscribed tumor of the radix of the tongue. The slides come from the tongue specimen.

Diagnosis: Cribriform adenocarcinoma of the tongue type (CATS)

References
CASE 5. A 53-year-old woman presented with a tumor of soft palate of 6 months duration

Diagnosis: Polymorphous low grade adenocacinoma (PLGA)

References
CASE 6. 63-year old man presented with rapidly growing tumor in the parotid gland. Grossly the tumor measured 6x5x2 cm. One cervical lymph node was involved by metastasis at the same time. The patient died two year later with multiple cervical lymph node and distant lung metastases.

Diagnosis: Salivary duct carcinoma

References

CASE 7. 26-year old woman presented with slowly growing tumor in the parotid gland. Grossly, the tumor measured 1.5 cm in diameter. No recurrence is noted 10 years after surgery.

Diagnosis: Pleomorphic adenoma with unusual features

References
CASE 8. 70-year old man presented with non-healing ulceration in molar region after extraction of the tooth 4 years earlier.

Diagnosis: Hyalinizing clear cell carcinoma of minor gland

References

CASE 9. A 76-year old woman presented with a rapidly growing tumor of parotid gland. Radical parotidectomy and cervical lymph node dissection of left side was performed, and followed by radiotherapy. The patient died after two years because of cancer dissemination (metastases in multiple lymph nodes and lungs). No autopsy performed.

Diagnosis: High grade transformation of acinic cell carcinoma

References
Margarida Lima graduated in Medicine at the Institute for Biomedical Sciences Abel Salazar (ICBAS), University of Porto (UP), at 1986. In 2004, she earned a Ph.D. in Medical Sciences from the UP, at ICBAS.

Positions/medical activity: At the Clinical Hematology Department of the CHP, she is responsible for the Cytometry Lab, since 1992, and for the Unit for Hematology Diagnosis, since 2011. She also assumes the co-responsibility for a Multidisciplinary Consultation for Cutaneous Lymphoma (CMLC), since 2003/2004. From 2005 to 2009, she was invited for the position of Medical Assistant of the Clinical Direction and she is the head of the Department of Teaching, Learning and Research of the Hospital since 2006.

Teaching activity: As a part of her medical activity and technical responsibility at the Cytometry Lab, Hematology consultation and CMLC from the CHP, she supervised more than 100 stages for medical, biomedical sciences and health technology students, as well as for medical doctors, lab technicians, nurses and other health professionals. She also orientated medical doctors in training for specialization in Clinical Hematology, Immunohemotherapy and Clinical Pathology. As invited professor at the ICBAS and other Portuguese faculties and universities, she has been responsible for teaching of classes, disciplines and modules from various doctoral, master and graduation courses. As professor, she supervised more than 30 doctoral theses, master dissertations and graduation projects, and integrated more than 15 academic and medical specialty juries. She also participated in the organization and was trainer in more than 20 courses.

Scientific and research activity: Her research interest centers on lymphoproliferative disorders, leukemia and lymphoma, mainly in those arising on T- and NK-cells. As part of her scientific activity she authored or coauthored more than 80 papers published in scientific journals with peer-review, most of them indexed in the MedLine, and more than 200 presentations in scientific meetings (lectures, oral communications and posters) and gave more than 50 invited lectures. She also integrated the organizing or scientific committees in various meetings and chaired numerous scientific sessions. As the head of the Department of Research of the CHP and in the context of her own research activity, she assumed the coordination two research groups, the “Clinical Research Group” and the group for investigation of “Blood, lymphopoietic and hematopoietic disorders”, affiliated in the Multidisciplinary Unit for Biomedical Research (UMIB), recognized by the Portuguese Foundation for Science and Technology. She also shares the responsibility for the coordination of the “Anemia physiopathology” research team of the hospital. Recently, she was invited to integrate the Portuguese ethical committee for clinical research.

Awards and fellows: In 1987, she was awarded as the best medical student. In 1986 and 1989, she received fellows for staging at the Pasteur Institute, Paris France. Along the years her team received 15 awards and honorable mentions for scientific publications and presentations in medical congresses.
Flow cytometry in the diagnosis of the mature lymphoid neoplasms: experience of the centro hospitalar do porto.

Over the last years, flow cytometry has gained an increasing importance in the diagnosis and characterization of the haematological malignancies. When combined with cytological and histological studies, immunophenotyping is of a great help to distinguish reactive processes from neoplastic diseases, as well as to accurately diagnose and categorize most of the mature lymphoid neoplasms. Besides its general application in the study of peripheral blood and bone marrow samples, its role in the investigation of fine-needle aspirates, serous effusions, cerebrospinal fluid and other body fluids has become more and more relevant as an important complement to the cytological diagnosis in these problematic biological samples. At the same time, cell suspensions obtained from core and surgical biopsies are used with increasing frequency for immunophenotyping, complementing the information obtained in the histological and immunohistochemistry studies of the different organs and tissues. In addition, cytogentic and molecular tests are helpful in identifying specific chromosomal and gene defects associated with particular disease entities or with prognostic significance.

Flow cytometry allows for an accurate identification of different B–cell reactive patterns observed in lymph nodes, such as follicular hyperplasia, whenever a typical reactive germinal centre B–cell immunophenotype is found; parafollicular hyperplasia can also be suspected based on the preferential expansion of otherwise normal activated CD4+ T cells. In addition, evidence for a polyclonal B cell expansion, with or without lymphoplasmacytic differentiation, and/or characteristic T- and/or NK–cell activation related patterns are observed in the peripheral blood from patients with reactive lymphocytosis.

Most mature B- and T– and NK–cell neoplasms that are recognized as independent disease categories in the World Health Organization (WHO) classification can be easily diagnosed by flow cytometry because of their unique phenotypic features. This applies to B–cell chronic lymphocytic leukaemia/small lymphocytic lymphoma, mantle cell lymphoma, follicular lymphoma and hairy cell leukaemia, among others. Diffuse large B cell lymphomas are also identify by simultaneously analysing the immunophenotype and the light scatter properties of the neoplastic B–cells; however, this particular group of mature B–cell neoplasms is very heterogeneous phenotypically, probably because they comprise several distinct pathological entities. The diagnosis of some disease entities by flow cytometry, such as the marginal zone B cell lymphoma is still of exclusion and the identification of other specific disease types, including B–cell prolymphocytic leukaemia and lymphoplasmacytic lymphoma, still relies on conventional morphological studies. For instance, the diagnosis of the former depends on the presence of prolymphocytes in the blood, whereas the latter is diagnosed mainly based on the morphological evidence of lymphoplasmacytic differentiation.

In that concerning plasma cell disorders, flow cytometry proved to be an important tool to identify and quantify, as well as to distinguish normal from monoclonal / phenotypically aberrant plasma cells. Using flow cytometry, we are able to discriminate monoclonal gammapathies of undetermined significance from multiple myeloma cases, to identify groups with different prognosis among patients with plasma cell myeloma, as well as to monitoring the response to therapy, at levels of minimal residual disease. Other plasma cell neoplasms, such as plasma cell leukaemia and plasmacytoma, are also easily diagnosed by flow cytometry.

In concerning mature T– and NK–cell neoplasms, flow cytometry allows for an accurate distinction between reactive (activated) and neoplastic T– and NK–cells in most occasions, based on the identification of activation–related or aberrant phenotypic profiles; in addition, the identification of clonally expanded T–cell populations can be facilitated by studying the repertoires of families of the T–cell receptor chains variable regions. T–cell prolymphocytic leukaemia, Sezary syndrome and T–cell large granular lymphocytic leukaemia are easily diagnosed by flow cytometry, especially if conjugated with clinical and morphological features. The same occurs for specific peripheral T–cell and NK–cell lymphoma subtypes with unique immunophenotyping profiles (e.g. HTLV–I associated T–cell leukaemia / lymphoma, angioimmunoblastic T cell lymphoma, and hepatosplenic T cell lymphoma, extranodal NK cell lymphoma, nasal type). In contrast, routine flow cytometry usually fails to diagnose Hodgkin lymphoma, as well as particular non–Hodgkin lymphoma subtypes, such as anaplastic large cell lymphoma, for which specific staining and/or acquisition flow cytometry protocols are requested in order to establish the diagnosis. Nevertheless, routine flow cytometry is useful to characterize the reactive lymphocytic infiltrate, which often has a very typical composition.

Herein we discuss the most relevant aspects concerning the use of flow cytometry in a clinic haematology laboratory, focusing on the potentials, advantages, limitations and pitfalls of this technique for the diagnosis, classification and monitoring of the tumours of the lymphoid tissues. Moreover, we present the experience of the Laboratory of Cytometry of the Centro Hospitalar do Porto in this area and describe the general procedures used in this Lab for sample collection, processing and cell staining. Finally, we summarize the main phenotypic features that characterize the mature lymphoid neoplasms, as defined by the 2008 WHO classification schema.
Pedro Farinha, M.D., Ph.D. is Staff Attending of the Anatomic Pathology Division, Centro Hospitalar de Lisboa Central (CHLC), Lisboa, Portugal and Assistant Professor at Faculdade de Ciências Médicas de Lisboa, Portugal.

He received his Anatomic Pathology Residency Training at the Portuguese Institute of Oncology, Lisbon, Portugal (1998–2003), with Residency Rotation (3 month) in Hematopathology at the British Columbia Cancer Agency (BCCA), Vancouver, BC, Canada (2001). He did Fellowship at Molecular Pathology in Oncology (Canadian Institute of Health Resources – CHIR) at BCCA, Vancouver, BC, Canada (2004–2006) and Fellowship at Clinical Hematopathology at BCCA (2006–2007). He has received several research or equivalent grants as principal or co-investigator.

Professor Farinha was engaged in Pathology teaching since 1998, became Assistant Professor in 2002 and since 2010 is co–organizing/teaching the module of Translational Research theoretical course and practical training.

He has been instructing residents of Anatomic Pathology since 2007, teaching in hematopathology rotations (3 months) to both Anatomic Pathology and Hematology residents.

Professor Farinha has made more than 60 presentations (more than 30 oral) in international scientific Meetings with referees, such as USCAP, ASH and EAHP annual meetings as well as ICML, Keystone Meetings and iwNHL. He was received seven awards. He also did a significant contribution to the medical literature with 31 published scientific articles. He is Journal Reviewer at several Medical Journals. He was oral Session Moderator & Session Reviewer at the 49th American Society of Hematology – Annual Meeting in Atlanta 2007 and is Co–organizer of the Annual Meeting of the European Association of Haematopathology / Society of Hematopathology taking place in Lisbon, October 2012.
1. **Follicular Lymphoma Microenvironment**

Until recently cancer biology has been focused on the study of the genetic alterations harboured by neoplastic cells that distinguished these cells from their normal counterparts. More recently, the concept of cancer ecosystem characterized by co-evolution of tumour clones vs. non-tumoral immune cells through complex, diverse and dynamic states with multiple variables, resulted in a growing appreciation of the contribution of the tumor microenvironment to the pathogenesis of tumours. In follicular lymphoma, the role of microenvironment has gained significant momentum with the evidence of an important clinical impact in cancer patients. Moreover, therapy regimens that target tumour microenvironment have shown significant and promising results thus being recently approved by FDA. The tumoral microenvironment encompasses the accessory and immune-related cells in the immediate vicinity of the tumour cells that engage in crosstalk and cell-to-cell contact with the neoplastic cells, typically providing a nurturing milieu by providing nutrients, new blood vessel formation and fostering immune privilege. The microenvironment may fluctuate between anatomic sites in a given B cell clonal proliferations, probably early in development of lymphoma with some sites seemingly creating a niche that promotes acquired drug resistance and cancer stem cell maintenance. Understanding the role of the microenvironment in lymphoid malignancies has also fostered renewed interest in the development of biological agents that act by disrupting the growth-promoting interactions or crosstalk between neoplastic and non-neoplastic cells, resulting in a potential paradigm shift in therapeutic strategies away from standard chemotherapy alone.

2. **The Pathology of Aggressive Non-Hodgkin Lymphomas**

Aggressive non-Hodgkin lymphomas comprise a large group of distinct lymphoid neoplasms, accounting for the majority of lymphomas we encounter in daily practice. The list of diseases included under this rubric have expanded recently due to the new genome molecular profiling studies. Distinguishing between these NHLs is frequently highly relevant for the patient, as both treatment and prognosis may be decisively affected. Importantly, there are new subtypes of lymphomas that are indistinguishable under the microscope but originate from lymphocytes that differ in development stage and that use distinct oncogenic programs. Different lymphoma subtypes will be discussed with emphasis on how to use ancillary techniques essential for a correct diagnosis.

**Notes**
CASE 1. 60 year-old man with night sweats and a right neck lymphadenopathy. Lymph node excisional biopsy.

Diagnosis: Composite lymphoma: diffuse large b cell lymphoma with low & high grade follicular lymphoma with associated follicular lymphoma in situ.

References

CASE 2. 38 year-old female with fatigue and an enlarged left axillary lymph node. She moved from Western Africa one years before the diagnosis. Lymph node excisional biopsy.

Diagnosis: HHV8–associated multicentric castleman disease.

References
CASE 3. Incidental right neck lymphadenopathy with 2cm in a 72 year-old female. Lymph node excisional biopsy.

**Diagnosis:** Angioimmunoblastic t cell lymphoma.

**References**

CASE 4. 82 year-old male with pancytopenia and hepatosplenomegaly. Bone marrow biopsy.

**Diagnosis:** Intravascular large b cell lymphoma.

**References**
CASE 5. Weight loss, night sweats and increased LDH in 64 year-old man. Enlarged retroperitoneal mass on CT scan. Retroperitoneal mass incisional biopsy.

**Diagnosis:** B cell lymphoma, unclassifiable with features intermediate between diffuse large b cell lymphoma and burkitt lymphoma.

**References**

CASE 6. 85 year-old man with a left axillary mass. Incisional biopsy.

**Diagnosis:** EBV positive diffuse large b cell lymphoma of the elderly.

**References**
CASE 7. Multiple lymphadenopathy in a 95 year-old female. Lymph node excisional biopsy.

Diagnosis: Aggressive systemic mastocytosis – lymphadenopathic systemic mastocytosis with eosinophilia.

References

CASE 8. 62 year-old female with weight loss, fatigue and pancytopenia. Bone marrow biopsy.

Diagnosis: T cell / histiocyte-rich large b cell lymphoma, micronodular variant.

References

CASE 9. 5cm jejunal mass in a 52 year-old man. Surgical jejunectomy with 30cm.

Diagnosis: Enteropathy-associated T cell lymphoma, Type II.

References
- Swerdlow SH et al. World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC
João Pires, M.D. is Consultant Radiologist at Centro Hospitalar do Porto and is subspecialized in Musculoskeletal Radiology. He graduated in Medicine by the Faculty of Medicine, University of Coimbra (2004). He received his Radiology Residency Training in Centro Hospitalar do Porto (2006–2011). In 2012 he earned the European Diploma in Radiology and the Diploma of the European Society of Musculoskeletal Radiology. Dr. João Pires has taught medical students in the disciplines of Clinical Anatomy (2006–2007) and Radiology (2006–2011). He has earned 3 scholarships. Dr. João Pires has 2 scientific publications and has made several presentations in National and International Scientific Meetings including 8 posters, 10 educational exhibits and 4 oral communications, receiving 2 Awards of Merit. He has attended 10 national and 11 international Radiology Congresses, received training in 13 advanced courses dedicated to the musculoskeletal field and other Radiology Courses including for e.g., the renowned American Institute for Radiologic Pathology Four Week Correlation Course (USA, 2011).

Pedro Cardoso, M.D., is Orthopaedic Surgeon Senior Consultant in Hospital de Santo António, Centro Hospitalar do Porto (C.H.P), Porto, Portugal. He graduated in Medicine at the Faculty of Medicine, Porto University (1987) and completed his Residency Orthopaedics Training at C.H.P. in 1996. He received training at the Rizzoli Orthopedic Institute (Bologna) and the Royal National Orthopaedic Hospital (Stanmore, London) in Musculoskeletal Pathology. As Orthopaedic Surgical Consultant at Hospital de Santo António, he devoted himself to an intense activity, integrating a multidisciplinary team of Musculoskeletal Pathology. Dr. Pedro Cardoso is Invited Assistant Professor of Orthopaedics in Abel Salazar Institute of Biomedical Sciences (ICBAS). He is Member of Section to the Bone Tumors Study of the Portuguese Society of Orthopaedics and Traumatology and was the Coordinator for the biennium 1999/2000. He has made significant contributions with numerous scientific articles and book chapters in Musculoskeletal Tumor Pathology including “O Joelho” – J. Espregueira Mendes, Pedro Pessoa, Lidel 2006, “O Ombro.” – A. Cartucho, J. Espregueira Mendes. Lidel 2009 and “Critérios Fundamentais em Fracturas e Ortopedia.” – Luís Serra, 3ª ed. Lidel 2012.”
Updates on the clinical and radiologic diagnosis of bone and soft tissue tumours

The presentation will focus on the role of imaging in musculoskeletal neoplasms, diagnostic procedures and treatment strategies, including new techniques. A brief overlook will be given of modalities used, information provided, limitations, pitfalls and recent advances. Radiologic-pathologic integration in certain problems (e.g. cartilage forming tumours, small round cell malignancies, giant cell tumours...) will be stressed. Although pathology is regarded as the gold standard, clinic-radiologic information and communication between specialties is crucial to reach a diagnostic consensus.

Notes
Medical Doctor (M.D.) degree: School of Medicine, Catholic University of Santiago of Guayaquil, Ecuador
Master of Science (M.Sc.) in Molecular Biology degree: Inter-University Post-Graduate Programme of Tropical Molecular Biology, Faculty of Sciences, Vrije Universiteit Brussel [Free University of Brussels], Brussels, Belgium
Anatomic and Clinical Pathology Residency Programs: Departments of Pathology and Laboratory Medicine, Yale University School of Medicine, Yale-New Haven Hospital, New Haven, CT
Graduate Professional Certificate in Health Care Management: School of Business, University of New Haven, New Haven, CT
Clinical Fellow in Pediatric Pathology: Harvard Medical School, Boston Children's Hospital, Boston, MA
Associate Professor and Director of Pediatric and Musculoskeletal Pathology: Department of Pathology, Yale University School of Medicine, New Haven, CT (until 12/2009)
Associate Professor: Department of Pathology, Medical College of Wisconsin, Milwaukee, WI (since 1/2010)
Director of Anatomic Pathology and Surgical Pathology: Froedtert Hospital and Medical College of Wisconsin, Milwaukee, WI
Associate Medical Director: Dynacare Laboratories, Milwaukee, WI
Associate Director: Anatomic and Clinical Pathology Residency Program, Medical College of Wisconsin, Milwaukee, WI
Vice-Chairman of Anatomic Pathology: Department of Pathology, Medical College of Wisconsin, Milwaukee, WI
Molecular and cytogenetic aspects of soft tissue and bone tumors

Mesenchymal tumors, as a whole, are rare neoplasms, which frequently require the application of complex molecular tests for their diagnosis. As more advanced testing is becoming increasingly available to a wider population, general surgical pathologists are in need of understanding basic principles of molecular pathology in order to be able to interpret complex text results, which may have direct implications in the diagnosis, prognosis and targeted treatment of bone and soft tissue tumors. As molecular pathology techniques and the tenets of personalized medicine are becoming widespread and common practice, both in the academic and the community settings, general surgical pathologists will increasingly be faced with having to interpret highly sophisticated molecular test results.

In this session we will deliver an in–depth review of state of the art molecular principles, and their application towards the diagnosis and targeted management of bone and soft tissue neoplasms. We will review basic principles in molecular biology of bone and soft tissue tumors, and the practical application of that knowledge through the appropriate ordering and interpretation of advanced molecular diagnostic tests. Attendants will also benefit from reviewing state of the art information related to personalized treatment and molecular targeting of specific tumors of bone and soft tissues.

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CASE 1. 62 year-old man presenting with left intra-abdominal “desmoid-type fibromatosis” recurring as high-grade sarcoma 5 years later.

Diagnosis: Dedifferentiated liposarcoma.
CASE 2. 93 year-old female patient presenting with 15 cm left medial thigh deep soft tissue mass.

**Diagnosis:** Well-differentiated liposarcoma with myxoid changes.
CASE 3. 14 year-old boy presenting with 1.9 cm “myxofibrosarcoma” involving superficial soft tissues of left upper arm.

Diagnosis: Nodular fasciitis.
CASE 4. 12 year-old girl with 5 x 2.5 cm soft tissue mass of left shoulder.

Diagnosis: Desmoplastic small round cell tumor.
Boštjan Luzar is a consultant pathologist and Professor of Pathology at the Faculty of Medicine, University of Ljubljana, Slovenia and Head of the Dermatopathology Section at the Institute of Pathology, Faculty of Medicine, Ljubljana, Slovenia. After finishing his residency, he has received additional training in dermatopathology at St John’s Institute of Dermatology, St Thomas’s Hospital, London. His contribution to scientific research and medical literature is demonstrated in over 60 publications in high quality peer reviewed scientific journals. In addition, he has co-authored three chapters in the latest edition of the book ‘McKee’s Pathology of the Skin with Clinical Correlations’. He has been committed to several invited lectures at the national and international levels, attesting his accomplishment and reputation in the field of Dermatopathology.
Problematic melanocytic lesions

Abstract
Most melanocytic lesions in children and adults do not differ histologically from similar types of lesions in the adult population. Nevertheless, exceptions do exist and a subset of melanocytic naevi in children and adolescents display architectural and cytological abnormalities. Such changes are not only age-related, they can also be influenced by their occurrence at special sites and under special circumstances, and must not be misinterpreted as melanoma. Essential clinical information thus includes the age of the patient and the site of occurrence.

This review focuses on peculiarities of melanocytic naevi in the first years of life, proliferative nodule in congenital naevi, pagetoid Spitz naevus, recurrent naevus, melanocytic naevus with Meyerson’s phenomenon, melanocytic naevus with a halo phenomenon, combined melanocytic naevus and deep penetrating naevus.

Key words: melanocytic naevi, children, adolescents, congenital melanocytic naevi, proliferation nodule, pagetoid Spitz naevus, recurrent melanocytic naevus, Meyerson’s phenomenon, halo naevus, combined melanocytic naevus, deep penetrating naevus.

Congenital naevi in neonates and young children
Congenital melanocytic naevi are traditionally defined as those present at birth and can be observed in 1 to 6% of neonates (1). Based on the size of the lesion in adulthood, they are categorized into small (less than 1.5 cm), medium-sized (1.5 to 20 cm) and large or giant congenital melanocytic naevi (more than 20 cm) (1). Lesions larger than 20 cm in size in adulthood correspond roughly to a size larger than 9 cm on the scalp and larger than 6 cm on the body of a neonate (2, 3). Large or giant congenital melanocytic naevi occur in about 1 in 20,000 births (4).

Clinical features
Congenital melanocytic naevi show a tendency for morphological changes over time. The initial lesion is usually a flat, evenly pigmented tan to black plaque or patch, which eventually becomes elevated and unevenly pigmented (1). Papules, nodules, as well as verrucous or cerebriform changes can subsequently develop, which might require histological examination to exclude melanoma. The majority of lesions contain increased numbers of terminal hairs. Congenital melanocytic naevi located on the scalp show a tendency to lighten and regress over time (1).

Histological features
The majority of melanocytic naevi in neonates and young children display histological features similar to those seen in ordinary or congenital melanocytic naevi (5-10). They can be junctional, compound or intradermal. The depth of the melanocytic infiltration is generally related to the size of the melanocytic naevus. Small melanocytic naevi typically show an extension along the adnexal structures and neurovascular bundles. While pure intradermal melanocytic naevi are rare in the first years of life, large congenital melanocytic naevi typically show diffuse extension into the subcutis. Maturation of the dermal melanocytic component is invariably present.

Occasionally, however, a subset of congenital melanocytic naevi in neonates and young adults display concerning histological features. They may lead to a mistaken diagnosis of melanoma, especially if the age of the patients is unknown. Two such settings are recognized and must not be confused with melanoma: an atypical melanocytic proliferation in the epidermis and a proliferation nodule or multiple nodules within congenital melanocytic naevi (see below).

Atypical epidermal melanocytic proliferation consists of large and abnormally located junctional nests as well as lentiginous and pagetoid proliferation of either single melanocytes or small groups of melanocytes. Melanocytes usually display an epithelioid morphology. Cytological atypia is present...
in up to 30% of cases and is usually mild but may on occasion be severe \(^{(1, 10)}\). Junctional mitoses can be frequent. Pagetoid proliferation is commonly present at the periphery of the lesion. The dermal melanocytic component is usually unremarkable. Nevertheless, cytological atypia may on occasion be observed in the dermal melanocytic component and is associated with impaired maturation. Variation in the amount of melanin pigment in the cytoplasm of melanocytes is common \(^{(10)}\). Mitotic activity is frequently absent. However, an occasional regular mitosis in the superficial dermal melanocytic component should not be regarded as a sign of malignancy.

**Differential diagnosis**
Malignant melanoma is exceedingly rare in neonates and young children. Importantly, however, melanoma in congenital melanocytic naevus usually develops within the dermal component and not in the epidermis, and shows similar histological features to melanoma in adults, i.e., lack of maturation, prominent mitotic activity, including atypical ones, expansile growth and pleomorphism of melanocytes.

**Proliferative nodule within a congenital naevus**
A proliferative nodule within a congenital melanocytic naevus was originally described by Reed et al in 1975 and further delineated as a specific entity by Clark et al in 1990 \(^{(11, 12)}\). A proliferative nodule is defined as a distinctive melanocytic proliferation in the dermis or rarely subcutis that develops in the background of a compound, most frequently a congenital melanocytic naevus. Although a proliferative nodule uniformly follows a benign clinical course, the histological features of this peculiar melanocytic proliferation are worrying and may lead to the incorrect diagnosis of an intradermal melanoma.

**Clinical features**
A proliferative nodule presents as a smooth surfaced nodule in the background of a congenital melanocytic naevus, most frequently although not exclusively within a giant congenital melanocytic naevus \(^{(13-20)}\). The lesion shows a predilection for the trunk and back, followed by the scalp and face. A proliferative nodule is usually already present at birth and shows female predominance. Solitary lesions are most frequent but satellite lesions can also be present. On occasion, a proliferative nodule may become ulcerated \(^{(20)}\). The natural history of proliferation nodules is one of spontaneous gradual regression. Alternatively, they can be a stable lesion over a prolonged period of time, or exhibit enlargement and hyperpigmentation.

**Histological features**
A scanning magnification demonstrates a well-defined nodular melanocytic proliferation in the superficial and middle dermis \(^{(13-20)}\). Much more rarely, the nodule can involve also the superficial subcutis. Increased cellularity within the nodule, as compared with the background melanocytic proliferation, is easily perceived. Although the lesion in the great majority of cases blends at its margin with the adjacent ordinary melanocytic proliferation, little or no blending may also be seen. A proliferation nodule is composed of epitheliod or spindled melanocytes that are larger than the neighboring melanocytes. Lesional melanocytes have abundant eosinophilic cytoplasm and generally display mild nuclear atypias. Intranuclear pseudoinclusions are frequent. Nucleoli are small and are usually not prominent. Mitoses are rare and regular. Their number is by definition less than 1 mitosis per mm\(^2\). Atypical mitoses and necrosis within the nodule are not a feature of a proliferation nodule. Maturation is present and is most easily found at the site and at the bottom of the nodule. Occasionally, greater nuclear pleomorphism and macronucleoli can be observed in a proliferation nodule, which is not associated with increased mitotic activity. A mild to moderate mononuclear inflammatory cell infiltrate composed of lymphocytes, confined
to the proliferation nodule, can be sometimes be present. Epidermal involvement is rare but can be present. No pagetoid spread is however seen. Areas of mesenchymal differentiation can occasionally be seen within a proliferative nodule. Although proliferation nodules generally show an expansile growth pattern with pushing borders, they may on occasion also demonstrate infiltrative but non-destructive growth, preserving hair follicles and eccrine ducts. Molecular studies on proliferative nodules have shown either no chromosomal aberrations or have demonstrated the presence of numerical anomalies of the whole chromosomes\(^{(21, 22)}\). Melanomas generally show more complex genetic aberrations, including gains and losses of chromosomal fragments\(^{(22)}\).

**Differential diagnosis**

Main differential diagnosis is with malignant melanoma (see above). Distinguishing histological features include the lack of uniform high-grade nuclear atypia, lack of necrosis within the nodule, rarity of mitoses (less than 1 per square mm) with the absence of atypical mitoses, presence of maturation, lack of pagetoid spread and absence of destructive expansile growth\(^{(20)}\).

**Pagetoid Spitz naevus**

Pagetoid Spitz naevus, also designated as intraepidermal Spitz tumour with prominent pagetoid spread, is a distinctive melanocytic proliferation originally described by Busam and Barnhill in 1995\(^{(23)}\). Pagetoid Spitz naevus is a morphological variant within the spectrum of epithelioid and spindle cell melanocytic naevi (e.g. Spitz naevi). It differs from a conventional junctional Spitz naevus by proliferation of single epithelioid melanocytes within the epidermis. Such proliferation is generally a minor component of a conventional junctional Spitz naevus. The main importance of pagetoid Spitz naevus lies in its distinction from melanoma in situ, early invasive melanoma and other non-melanocytic proliferations with pagetoid growth pattern (see below).

**Clinical features**

Pagetoid Spitz naevus usually presents as a solitary symmetrical flat or slightly elevated light to dark brown macule measuring less than 4 mm in maximum diameter. It shows a predilection for young females (mean age about 25 years) and occurs most frequently on the lower extremities\(^{(23-25)}\).

**Histological features**

Low power magnification generally demonstrates a well-circumscribed, well-demarcated and usually symmetrical proliferation of melanocytes within the epidermis\(^{(23-25)}\). By definition, the lesion is small in size and is composed of a proliferation of single epithelioid melanocytes. Furthermore, the morphology of epithelioid melanocytes is fairly uniform within the lesion. Although the single cell proliferation of melanocytes is the predominant growth pattern, junctional nests can also on occasion be seen. In such instances, melanocytic nests form a minor melanocytic component. The melanocytes are round, oval or polygonal and have well-defined cell borders. The cytoplasm is abundant and glassy. The nuclei of melanocytes are frequently larger than the nuclei of keratinocytes, are round to oval, and contain prominent eosinophilic nucleoli. Nuclear chromatin is finely granular. Each cell is typically separated from the neighbouring cells by a retraction artefact. Although pagetoid proliferation is usually limited to the lower half of the epidermis, full thickness pagetoid spread can also be present. Mitoses are generally absent. Additional changes frequently seen in pagetoid Spitz naevus include acanthosis of the epidermis and mild mononuclear inflammatory cell infiltrate composed of lymphocytes and histiocytes within the superficial dermis.

**Differential diagnosis**

The main differential diagnosis includes melanoma *in situ*. Low power examination of melanoma...
in situ demonstrates an asymmetrical and poorly demarcated melanocytic proliferation that usually extends over a larger surface area of the skin. Cytological atypia is non-random, is more pronounced and widespread, upward migration of atypical melanocytes is often multifocal. Fine or dusty melanin pigment and mitoses are also suggestive of melanoma. In addition, melanoma in situ frequently shows a nesting pattern of melanocytic proliferation with irregularly sized nests, dyscohaesion of nests and their confluence. Non–melanocytic intraepidermal pagetoid proliferations, such as mammary/extramammary Paget’s disease, pagetoid squamous cell carcinoma in situ, sebaceous carcinoma in situ or intraepidermal lymphoid proliferations can be distinguished from melanocytic proliferations by negative S100 immunohistochemistry.

**Note of caution**
The diagnosis of pagetoid Spitz naevus in the elderly should always be questioned. If the diagnosis of pagetoid Spitz naevus is in doubt, a conservative re-excision is advised to ensure complete removal of the lesion.

**Recurrent melanocytic naevus**
Recurrent melanocytic naevus or so–called Ackerman’s pseudomelanoma is defined as an atypical melanocytic proliferation at the site of previous incomplete excision of a benign melanocytic naevus (26). Recurrent melanocytic naevus most commonly develops after shave biopsy, but similar lesions have also been reported following laser treatment, cryotherapy or local application of topical agents (27, 28). Nevertheless, it should always be taken into consideration that recurrent dysplastic melanocytic naevus or melanoma in situ can mimic a recurrent melanocytic naevus.

**Clinical features**
Recurrent melanocytic naevus most commonly develops within 6 months following the initial procedure, shows female predominance and most frequently occurs on the back (29). It presents clinically as an irregular, variably pigmented macular lesion at the site of a previous procedure (26).

**Histological features**
Defining histological features of a recurrent melanocytic naevus are architectural and cytological abnormalities restricted to the area above the scar. Abnormalities do not extend beyond this area and are usually limited to the epidermis. The lesion shows asymmetry, but generally shows good lateral circumscription. Lentiginous proliferation of individual melanocytes or small groups of atypical melanocytes are seen. In addition, focal nesting of melanocytes can also be present. Melanocytes frequently display hyperchromatic nuclei. Atypia of melanocytes varies from absent to severe and is most commonly mild to moderate (30). Focal pagetoid spread of melanocytes is occasionally seen and is usually restricted to the central part of the lesion. Mitoses are not present, or are very rare and regular. In the dermis, a superficial chronic inflammatory cell infiltrate with numerous macrophages is frequently present. A rete ridges pattern overlying the scar is frequently effaced. On occasion, however, retiform epidermal hyperplasia confined to the area of the scar can be seen (29). Residual junctional or more commonly intradermal melanocytic naevus is frequently present in association with the scar and shows unremarkable histological features.

**Differential diagnosis**
Clinico–pathological correlation is essential, and the previous biopsy, if available, should always be reviewed. A diagnosis of recurrent melanocytic naevus can be particularly challenging in the
absence of clinical information or slides from the previous biopsy. The most useful distinguishing features for recurrent melanocytic naevus are a sharp restriction of cytological and architectural abnormalities to the area of the epidermis above the scar, the absence of mitotic activity and the presence of the scar in the dermis.

Pseudomelanoma phenomenon has also been reported in a subset of melanocytic naevi, so called sclerosing naevi (31). They are characterized histologically by a central area of scar, not related to previous procedure or trauma, accompanied by remnants of a naevus at the periphery of the scar. Similar to pseudomelanoma phenomenon in recurrent naevi, the epidermal component in sclerosing naevi consists of irregularly sized and confluent nests of melanocytes with occasional upward extension of melanocytes, confined to the area above the scar. No significant atypia of melanocytes is seen and mitoses are absent or scarce. These changes are thought to be related to partial regression of the lesion.

**Meyerson’s phenomenon in melanocytic naevus**

Meyerson’s phenomenon is an annular dermatitis or eczema superimposed on a melanocytic lesion. It has most frequently been observed in association with an acquired, usually compound naevus (32-35). This phenomenon has also been reported, albeit rarely, in congenital melanocytic naevi and dysplastic naevi (36-38). The etiology of Meyerson’s phenomenon is at present unknown. Treatment with interferon has been implicated in the development of Meyerson’s phenomenon in isolated cases.

**Clinical features**

Meyerson’s phenomenon in melanocytic naevus presents as an asymptomatic or sometimes pruritic, erythematous lesion with a scaly border measuring up to 1.0 cm in diameter. Meyerson’s phenomenon shows a predilection for melanocytic naevi located on the trunk and proximal extremities, displays a male predominance, and is most commonly seen in the third decade (32, 33). Multiple lesions can also be seen. Following resolution of the dermatitis, the naevus appears unchanged. Areas of hypopigmentation can occasionally be seen.

**Histological features**

The defining histological features of Meyerson’s phenomenon include parakeratosis, acanthosis and spongiosis associated with a superficial perivascular chronic inflammatory cell infiltrate. The inflammatory cell infiltrate consists predominantly of CD4+ lymphocytes, both in the epidermis and dermis (36, 37). Eosinophils are sometimes conspicuous. There is no histological evidence of regression. Similar changes surrounding a diverse range of lesions, including seborrheic keratosis, stucco keratoses, keloids, naevus flammeus, squamous cell carcinoma, basal cell carcinoma, and dermatofibroma have occasionally been described (40, 41).

**Differential diagnosis**

Prominent eczematous reaction overlying melanocytic proliferation can obscure the true nature of the lesion. It is important to note that superimposed Meyerson’s phenomenon does not change the morphology of a melanocytic lesion. Although some degenerative atypias of melanocytes can be seen, the mitotic activity of melanocytes remains unchanged. Since such a phenomenon has also been reported in dysplastic melanocytic naevi and can be seen in melanoma in situ or invasive melanoma, careful evaluation of the underlying melanocytic lesion is of utmost importance.

**Halo Naevus**

Halo naevus was originally described by Sutton in 1916 and designated as leukoderma acquisitum
Halo naevus is a descriptive designation for a melanocytic naevus showing a distinctive clinical feature: a halo surrounding the melanocytic naevus. However, since a clinical halo can on occasion be missing, the use of the expression 'melanocytic naevus with halo phenomenon' has recently been proposed thus referring to the characteristic histological features.

The halo phenomenon is the consequence of a prominent immune host response in the dermis, which is directed towards melanocytes and eventually results in the regression of the lesion. The immune host response can on occasion be so extensive that it obscures the melanocytic nature of the lesion. Furthermore, lesional melanocytes can display prominent degenerative atypia, which may be mistaken for melanoma. Since the halo phenomenon is a non-specific reaction pattern, it can be found in a myriad of melanocytic proliferations, such as congenital melanocytic naevus, blue naevus, Spitz naevus, dysplastic naevus and even malignant melanoma.

Clinical features
The halo naevus is characterized clinically by a central macule or slightly elevated brown to dark lesion surrounded by a well-circumscribed area of hypopigmentation or depigmentation. The central area can be covered by scale or crust and have a verrucous appearance, especially in children. The halo naevus typically presents in the first two decades of life, shows a predilection for the trunk, especially back and has an equal sex distribution. The lesions can be multiple and occasionally show familial clustering. Patients with Turner’s syndrome have an increased prevalence of halo naevi as compared to the general population.

Histological features
Low power magnification can show significant architectural distortion. The lesion may be asymmetrical due to prominent lymphocytic infiltration. Melanocytic naevus is most frequently compound, and much more rarely purely intradermal or junctional. Junctional nests of melanocytes can be obscured by a lichenoid infiltrate. No pagetoid spread of melanocytes is generally seen. Cytological atypia is frequently seen in both junctional and dermal melanocytes, is usually mild to moderate and degenerative in nature. The dermal melanocytic component shows maturation. Mitoses are generally absent. Mitoses in the inflammatory cell component that are mistaken for melanocytes are an important diagnostic pitfall. In later stages of the lesion, melanocytes can be difficult to identify without additional S100 immunostaining. A completely resolved naevus is characterized by epidermal hypopigmentation associated with scattered dermal melanophages. Mild scarring may sometimes be evident.

By immunohistochemistry, the majority of lymphocytes are of a suppressor/cytotoxic T-cell phenotype, admixed with a minor population of CD4-positive T helper cells, B lymphocytes, macrophages and Langerhans’ cells. Occasionally plasma cells can also be seen. Pigment containing macrophages can be prominent, especially in the later stages of the lesion.

Differential diagnosis
Malignant melanoma can be distinguished from melanocytic naevus with halo phenomenon by the presence of melanoma in situ in the epidermal melanocytic component. Furthermore, the dermal melanocytic component in melanoma is characterized by non-random cytological atypia, the presence of dermal mitoses, lack of maturation, and expansile growth. Additionally, patchy inflammatory cell infiltrate and the presence of true regression (e.g., vascular proliferation, fibrosis) is also suggestive of melanoma.

Combined melanocytic naevus
Combined melanocytic naevus is defined histologically as a melanocytic naevus harbouring two or more distinct populations of melanocytes within a single lesion. A new designation, ‘melanocytic
naevus with phenotypic heterogeneity', has recently been proposed for such melanocytic lesions (10). Accordingly, a combined melanocytic naevus can display any combination of common melanocytic naevus (common acquired naevus, congenital naevus and dysplastic naevus), blue naevus (common, cellular and deep penetrating) and/or Spitz naevus (conventional or desmoplastic).

Clinical features
Clinical features of a combined melanocytic naevus can be unusual and concerning. A combined melanocytic naevus presents as a flat or eccentrically raised macule or papule showing variegated pigmentation. Sex distribution is equal and the lesion shows a predilection for the trunk, followed by head and neck, upper and lower extremity, perineum and buttocks (59). Combined melanocytic naevi have also been described at mucosal sites.

Histological features
The combination of deep penetrating naevus and common acquired melanocytic naevus appears to be the most frequent association (59-61). The cells of the common naevus generally occupy the upper part of the lesion, including the junctional component. Although different components are usually relatively well separated from each other, they may also be intermixed, thus making distinction between different melanocytic populations difficult (for example, common or cellular blue naevus and deep penetrating naevus).

Differential diagnosis
Careful examination of the lesion will demonstrate different melanocytic populations with distinctive histological features. Importantly, a lack of maturation can be seen in a subset of blue naevi (cellular and deep penetrating naevi). In addition, occasional regular mitoses in the dermal melanocytic component are not uncommon. Atypical mitoses are, however, not a feature in combined melanocytic naevi.

Malignant melanoma can be distinguished by the presence of melanoma in situ in the junctional component. Pure intradermal melanomas are rare and metastatic melanoma must be ruled out in such instances. The dermal melanocytic component usually demonstrates nuclear pleomorphism, an expansile growth pattern and mitoses, including atypical ones.

Deep penetrating naevus
Deep penetrating naevus may simulate melanoma both clinically and histologically. One or more disturbing histological features can frequently be found in deep penetrating naevus, and include asymmetry, plump but fairly regular nests of melanocytes in the dermis, cytologic atypia with nuclear pleomorphism, somewhat prominent eosinophilic nucleoli, an absence of maturation, the occasional presence of regular dermal mitoses and a patchy mononuclear inflammatory cell infiltrate. Although unusual, such histological features should not be regarded as a sign of malignancy in deep penetrating naevus. Deep penetrating naevi, on the basis of lack of maturation, prominent pigmentation, growth along neuro-vascular bundles and skin adnexa, and occasional presence of dendritic cells, have been regarded as a variant of a blue naevus (62, 63). However, the exact histogenesis of deep penetrating naevus has not to date been elucidated.

Deep penetrating naevus is a distinctive melanocytic naevus originally described by Seab et al in 1989 (64). A plexiform spindle cell naevus subsequently reported by Barnhill et al has essentially the same histological features (65, 66). In addition, a superficial variant of deep penetrating naevus lacking a deep penetrating component has been designated melanocytic naevus with focal atypical epithelioid components or clonal naevus (67, 68). Although the name ‘deep penetrating naevus’ implies extension into the deep dermis or subcutis, it is rather the combination of growth pattern and cell morphology that define the entity.
**Clinical features**

Deep penetrating naevus generally presents in the third decade of life as a solitary papule or nodule of less than 1 cm in the diameter, and shows a slight female predominance (F:M=1.3:1) \(^{60, 61, 64, 65, 68, 69}\). Less than 5% of deep penetrating naevi develop after the age of 50 years. A diagnosis of a deep penetrating naevus in elderly patients should be made with caution, e.g., by excluding a deep penetrating naevus–like melanoma (see below). The duration of the lesion prior to excision has been from recent development to several years and a congenital occurrence has also been documented \(^{60, 61}\). Pigmentation can be variegated, from light brown to black, but most lesions are darkly pigmented. Especially if a part of a combined melanocytic naevus, the lesion may appear asymmetrical and unevenly pigmented, thus raising the clinical suspicion of melanoma. The most frequent site of origin is the skin of the head and neck, followed by the extremities and trunk. Deep penetrating naevus has not been described on the palms and soles.

**Histological features**

The distinctive morphological features of a deep penetrating naevus can already be perceived with scanning magnification. Deep penetrating naevus is sharply demarcated, well circumscribed, often symmetrical and usually shows a wedge shaped configuration of the lesion with the base towards the epidermis and the tip towards the reticular dermis/subcutis \(^{62}\). Frequently, however, one or more extensions along the skin adnexa or neuro-vascular bundles into the deep reticular dermis and/or subcutis are seen, giving the lesion a plexiform appearance.

A limited junctional melanocytic component is present in 60–85% of deep penetrating naevi \(^{60, 61, 64, 66, 68, 69}\). The epidermal melanocytic component consists of lentiginous proliferation and nests of melanocytes with uniform round to oval nuclei, indistinct nucleoli and scarce to moderately abundant cytoplasm containing mild to moderate amounts of melanin. Focal pagetoid proliferation of uniform melanocytes into the uppermost layers of the epidermis has been very rarely reported in deep penetrating naevi \(^{61}\) but this feature is exceptionally found and should prompt careful scrutinizing of the lesion to exclude melanoma. The papillary dermis is frequently not involved, thus representing a tumour–free zone between the junctional melanocytic component, if present, and the melanocytic proliferation in the reticular dermis and subcutis. Discontinuity of the lesion in the papillary dermis, if present, may be a useful histological feature in excluding melanoma, which usually grows in continuity between the epidermis and the dermis.

The dermal component, usually located in the reticular dermis, consists of loose nests and vertically oriented fascicles of epithelioid cells and short spindle–shaped melanocytes. While the epithelioid morphology is more frequent in upper parts of the lesion, the spindle cell morphology may predominate in the deeper parts. The epithelioid cells differ from those seen in common acquired melanocytic naevi by displaying more abundant cytoplasm and larger nuclei. The proportion of both epithelioid and spindle–shaped components can vary greatly within and among the lesions. A confluence of nests may be seen, especially in the upper dermis and central areas of the lesion but is usually a focal feature. Discohesion of melanocytes is usually present at the periphery and bottom of the lesion, where melanocytes are seen splaying collagen fibres. Melanocytes grow along skin adnexa and neuro–vascular bundles without destroying them. Perineural extension is a common feature. Infiltration of the arrector pili muscle is a frequent occurrence in deep penetrating naevi.

Cytologically, melanocytic nuclei are hyperchromatic, with variation in their size and shape from round to oval. Nuclear contours may be irregular. Mild to moderate nuclear pleomorphism is a constant feature in deep penetrating naevi and may be focally marked, with the size of melanocytic nuclei being more than twice the size of basal keratinocytes. Considerable variation
in the size of the nuclei can also be observed within individual melanocytic nests and/or fascicles. No obvious maturation of melanocytes is seen. Cytoplasmic nuclear (pseudo)inclusions can usually be found. Nucleoli are small to medium sized and eosinophilic. Mitoses can be present, even in the deeper parts of the naevus. Their number ranges from 0–1.2 per mm². Proliferative activity in deep penetrating naevi is present in fewer than 5% of dermal melanocytes. Atypical mitoses are not found and their presence should arouse the suspicion of melanoma. Melanocytes have moderate to abundant cytoplasm, which is lightly to moderately pigmented. Areas of clear cell change within deep penetrating naevi are common. Other constant morphological components in deep penetrating naevi are melanophages and a mononuclear inflammatory cell infiltrate. Melanophages can be sparse and focal to abundant and dispersed throughout the lesion. They usually surround individual nests and bundles of melanocytes and are especially prominent at the periphery of the lesion. A mononuclear inflammatory cell infiltrate, composed predominantly of mature lymphocytes can be sparse and focal or prominent and diffuse, a pattern reminiscent of ‘tumour infiltrating lymphocytes’ in melanomas. In a subset of deep penetrating naevi, scattered pigmented dendritic melanocytes can be seen and, in this context, distinction from a variant of blue naevus may be difficult and has prompted some to suggest that deep penetrating naevus is part of the spectrum of blue naevus.

‘Atypical’ histological features in deep penetrating naevi
One or more histological features can frequently be found in deep penetrating naevi, which may cause concern as to the biological potential of the lesion. These include asymmetry of the lesion, expansile melanocytic nests in the dermis, random cytological atypia with nuclear pleomorphism, prominent eosinophilic nucleoli, absence of maturation, presence of dermal mitoses and mononuclear inflammation. Although unusual, such histological features are expected to be seen in about 40% of deep penetrating naevi and should not be regarded as a sign of malignancy. Random cytological atypia, with individual nuclei displaying enlargement and hyperchromatism, is present in a number of cases, particularly in younger individuals. This is not usually associated with an increase in mitotic activity.

Melanocytic naevus with focal atypical epithelioid component (clonal naevus) – a superficial variant of deep penetrating naevus
In 1994, Ball and Golitz described a series of 73 cases of a peculiar melanocytic naevus, characterised histologically by poorly circumscribed small groups or nests of large, deeply pigmented epithelioid melanocytes located in the superficial dermis, developing in the background of an ordinary melanocytic naevus. These melanocytes were distinguished by irregular nuclear contours, small nucleoli and prominent cytoplasm containing melanin. Mitoses were absent or extremely rare. Nests of melanocytes were surrounded by deeply pigmented melanophages. Subsequent study has confirmed that melanocytic naevi with focal atypical epithelioid component share a similar age and anatomic distribution with deep penetrating naevi. Furthermore, cytological features between the two entities were essentially identical – the only difference between the two entities being the depth of extension of the epithelioid component. Melanocytic naevus with focal epithelioid component has therefore been regarded as a superficial variant of a deep penetrating naevus.

Biological potential and treatment
Deep penetrating naevus is a benign melanocytic proliferation. In reviewing the literature of more than 300 deep penetrating naevi published to date, only three local recurrences following incomplete excision have been reported. Conservative and complete local excision appears to be the best treatment option for deep penetrating naevi.
Differential diagnosis

The main differential diagnosis is with melanoma. Primary cutaneous melanoma is an asymmetrical and poorly circumscribed proliferation of melanocytes with a junctional component comprising lentiginous and/or pagetoid proliferation of atypical melanocytes with destructive growth in the dermis. Discontinuity of the lesion in the papillary dermis, as frequently observed in deep penetrating naevi, is generally not seen in melanomas. The dermal melanocytic component in melanomas consists of expansile nests or sheaths of atypical melanocytes. Cellular atypias in melanomas are more pronounced than in deep penetrating naevi and are non–random in distribution. Furthermore, mitoses in melanomas are frequent, including atypical forms. Mitoses can also be observed in the deeper parts of the lesion. Metastatic intradermal melanoma can be separated from deep penetrating naevus by a wedge shaped configuration, symmetry and lack or very limited mitotic activity with absence of atypical mitoses of the latter. Pigment synthesizing melanoma is characterised by dome shaped proliferation of heavily pigmented epithelioid and spindle melanocytes with abundant cytoplasm, large vesicular nuclei and prominent nucleoli. Nuclear pleomorphism is usually apparent, mitoses can be found and also include atypical ones. Melanocytes show variable extension along the skin adnexa but also show more diffuse and destructive growth in the dermis, with frequent infiltration into the subcutis. The junctional component in pigment synthesizing melanomas, if present, is commonly dendritic.

References


Notes
CASE 1. A 60-year-old male with slowly growing polypoid lesion on the upper thigh

**Diagnosis:** Cutaneous (intradermal) clear cell sarcoma

**References**
- Reeves BR, Fletcher CD, Gusterson BA., *Translocation t(12;22)(q13;q13) is a nonrandom rearrangement in clear cell sarcoma*, Cancer Genet Cytogenet 1992; 64: 101–103
CASE 2. A 21-year-old female with a nodule on the nose

**Diagnosis:** Cellular neurothekeoma

**References**
CASE 3. A 69-year-old male with the lesion on the scalp. Clinical diagnosis: scar

**Diagnosis:** Desmoplastic melanoma

**References**
- George et al., *Subclassification of desmoplastic melanoma: pure and mixed variants have significantly different capacities for lymph node metastasis*, J Cutan Pathol 2009; 36: 425–432.
CASE 4. A 62-year-old male with the lesion on the upper arm

Diagnosis: PEComa (Perivascular epithelioid cell tumour)

References
CASE 5. A 74-year-old male with the lesion on the scalp.

**Diagnosis:** Atypical fibroxanthoma

**References**
CASE 6. A 21-year old woman in the 6 month of pregnancy with an atypical pigmented lesion on the vulva. Clinical diagnosis: melanoma

Diagnosis: Atypical compound genital melanocytic naevus

References
- Quddus M, *Atypical melanocytic nevi of genital type: A distinctive pigmented lesion of the genital tract often confused with malignant melanoma*, Dermatology Online Journal 16 (2): 9
Abílio Reis, MD, is Senior Consultant of Internal Medicine and Head of the Pulmonary Vascular Unit, Medicine Department, Hospital Santo António, Porto, Portugal. Professor Abílio Reis was invited Associated Professor of Medicine, Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal. He founded and coordinates the Study Group for Pulmonary Vascular Diseases, Portuguese Internal Medicine Society. Dr. Abílio Reis is consultant of the Portuguese Directorate-General of Health to organize the PAH National Health Care Network. He was chairman of the working group for the national PAH guidelines.
Update on pathology of Pulmonary Hypertension

Pulmonary Hypertension (PH) is a haemodynamic and pathophysiological state that can be found in multiple clinical conditions (more than thirty). These have been grouped in six clinical categories in accordance with the underlying clinical condition.

Group 1 PH or Pulmonary Arterial Hypertension (PAH) brings together diseases sharing characteristic pathologic lesions: sustained pulmonary vasoconstriction, lumen obliteration of small and medium size arteries and arterioles in association with the formation of plexiform lesions and in situ thrombosis, as well as concentric thickening of pulmonary arteries resulting from intimal fibrosis and medial hypertrophy. This structural damage of pulmonary arteries provokes an increase in pulmonary vascular resistance, right ventricular overload and finally right heart failure.

PAH is an infrequent disease having an estimated incidence around 2.4 cases/million adult population/year and prevalence between 15 and 50 subjects per million habitants. Detection and diagnosis of PAH is frequently delayed for 2 or 3 years with important implications on patients’ outcome. Its historical poor prognosis has been changing in the last decades with the discovery of new drugs and strategies for its treatment. Early detection and aggressive goal oriented therapy have brought improvements in the functionality, quality of life and survival of these patients.

PAH clinical management is complex and costly reason why patients must be oriented and managed in dedicated referral centres integrating a well-regulated national network.

Notes
Giovanni Falconieri is Senior Staff Pathologist at the University Hospital in Udine, Italy, where he is mainly involved in case sign-out supervision, outside consultation, and review of difficult and controversial cases. He graduated at the University of Trieste School of Medicine, Italy, where he also completed his Pathology Residency Program obtaining specialty certification in 1981. He is also certified in Forensic Medicine. Dr. Falconieri has received pathology training at the University of Miami School of Medicine in 1987–1988. Dr. Falconieri is a general surgical pathologist, although he has devoted special interest in thoracic and mediastinal pathology, especially lung cancer, pleural tumors, thymoma and thymic carcinoma. He has also a background in post mortem investigations, having served till 1996 as Clinical Professor of Pathology and Pathology Attending at the University of Trieste, one of the busiest autopsy facility of the world where an average of 2500 autopsies/year are pursued. He is the author of more than 100 papers, most of which indexed in the Pub Med. His contributions address pleural and mediastinal tumors, lung carcinoma and, in recent times rare skin tumors. Dr Falconieri has been appointed as ad hoc reviewer for several international, top rated international pathology journals such as Histopathology and Virchow’s Archives, and is currently in the Editorial Board of Annals of Diagnostic Pathology.
Overview of Pleural Mesothelioma

Malignant Mesothelioma

Although relatively infrequent, mesothelioma is the most common malignant tumor in the pleura but also the one that poses more difficulty in the diagnosis due to its varied histopathological appearance and related medicolegal issues. Their incidence in the United States is of approximately 3 to 7 cases per million persons in the population per year, and 15–20/million worldwide. Although mesotheliomas have been associated to the exposure of asbestos fibers, approximately 50% of individuals affected by mesotheliomas do not disclose an asbestos exposure, nonetheless a multifactorial etiopathogenetic mechanism may be claimed. In general mesotheliomas are more common in adult individuals the fifth-sixth decade. In those cases in which the tumor is associated with asbestos, the patient had been exposed for over 15 years to the asbestos fibers. It would be very unusual to find a case in which the tumor is linked to asbestos in a patient with only a few years of exposure.

Clinical Features

Clinical and radiological information play a highly important role in the diagnosis of mesothelioma. One of the most important aspects in the diagnosis of mesothelioma is the radiological evaluation, either a plain chest radiograph or more sophisticated studies such as computerized tomography. The following aspects should be inquired in cases suspicious of mesothelioma: nodular studding of pleura, diffuse thickening of pleura, encasement of the lung, unilateral or bilateral pleural involvement. Many times the clinical and radiological aspects of the cases are clear but the available material for histopathological examination is not enough to render a diagnosis of mesothelioma. In such circumstances, one should not commit himself/herself but rather raise the level of suspicion and request additional material if clinically indicated. The rationale is the fact that the surgical treatment for cases of mesothelioma can be very radical (i.e. extrapleural pneumonectomy), thus, the pathologist must be absolutely sure about the diagnosis. In addition current chemotherapy protocols are of questionable efficacy, hence delays in diagnosis are not as detrimental to patients as in other malignancies. Furthermore, it is well known that there are other pleural conditions of an inflammatory nature that may clinically and radiologically mimic malignant mesothelioma. Therefore, one should use the clinical and radiological information not to make a diagnosis per se but rather to orient oneself in the plan to follow with the use of immunohistochemistry and/or electron microscopy. Ultimately the diagnosis of mesothelioma is a pathological one and not a clinical or radiological diagnosis alone.

Pathological Features

Macroscopically mesotheliomas are tumors with a characteristic gross appearance. The tumor are most often diffuse though in some cases the neoplastic growth follows the intrapulmonary septa; in rare instances the tumor may involve the outer lung tissue subjacent the pleura. However, the presence of a well defined tumor mass in the periphery, even if the tumor also shows diffuse pleural involvement, should alert about the possibility of a peripheral lung cancer with diffuse pleural spread (i.e. pseudomesotheliomous cancers)

Histopathologic characteristics: Mesotheliomas may show several microscopical patterns. However, traditionally mesotheliomas have been divided into three categories: Epithelial, Sarcomatoid, Biphasic (combination of epithelial and sarcomatoid).

A. Epithelial mesothelioma is in our experience the most common variant. In addition to the classic tubulopapillary form, additional variants include the epithelioid, decidual, clear cell, glandular, or rarely the myxoid or adenomatoid subtypes. The variants of epithelioid mesothelioma are relatively uncommon and represent diagnostic challenges.
Special Studies

Immunohistochemistry has virtually replaced histochemistry and is the most used and often abused ancillary technique. Probably, in not so many field of surgical pathology immunohistochemistry of mesothelioma has been the subject of so many published studies, in particular addressing the positive identification of mesothelioma cells. Other studies have attempted at the recognition of adenocarcinoma as to properly rule out the presence of mesothelioma. Thus, the diagnosis of mesothelioma has in the past been considered one of exclusion. Many antibodies have been stated to be helpful in the diagnosis of mesothelioma, the fact is that only a few are use in practice. Currently, the most popular markers include keratin broad spectrum, keratin 5/6, calretinin, Leu-M1, CEA, B72.3, and Ber-ep4. Some of those markers have been stated to positively stain mesothelial cells (keratin 5/6 and calretinin) while other have been stated to identify cases of adenocarcinoma (CEA, Leu-M1). In the setting of an epithelial mesothelioma these markers are more important. However, their positive staining does not constitute a full proof diagnosis of the tumor. For instance, depending on the antibody use, it may alter the interpretation of it. One of those cases is CEA, which has been demonstrated to show some positivity in about 5% of cases of mesothelioma. However, it is possible that this phenomenon may be explained by the use of unabsorbed heteroantisera to CEA. The use of monoclonal CEA appears to be more reliable in this evaluation. In short, we can summarize the immunohistochemical studies in the following manner: if the tumor in question shows positive for either of these antibodies – broad-spectrum keratin, keratin 5/6, and calretinin, then the most likely interpretation is that of mesothelioma. However, if there is positive staining for either CEA, le-M1, B72.3 or other carcinomatous epitope, then the diagnosis should lean more towards adenocarcinoma.

Differential Diagnosis

In the setting of an atypical epithelial cellular proliferation the most important conditions to establish that it is malignant first, the next step being the assessment of tumor cell origin. The single, most reliable microscopic criteria is the documentation of tumoral infiltrate in the adipose or soft tissue of the chest wall or the subpleural pulmonary tissue. As a corollary, in superficial biopsy fragments the diagnosis of mesothelioma should not be rendered with certainty as long as mesothelial hyperplasia may not be ruled out. In addition, cellular proliferation in surface, granulation tissue and fibrin should prompt a diagnosis of reactive/inflammatory lesion. Mitoses and atypia may be observed in both mesothelioma and reactive hyperplasia, hence are not useful morphologic criteria. If one has concluded that the cellular proliferation in question is malignant, then the use of immunohistochemical studies, namely the use of the above listed carcinomatous antibodies is the next step. Similar steps are adequate in the event of a metastatic epithelial neoplasm from other source to the pleura.

B. Sarcomatoid Mesothelioma:

This variant of mesothelioma is less common than the epithelial one and probably represents less than 15% of all these tumors in its pure form, however it poses more diagnostic challenges. The tumor characteristically has a growth pattern of spindle cells with an elongated nuclei and inconspicuous nucleoli and mimicks a sarcoma of soft tissues. A fibrous, sometimes collagenized ground substance may be observed as in desmoplastic variant of spindle cell mesothelioma. In rare instances, metaplastic bone or cartilage forming tumors have been described. Another variant is the so called lymphohistiocytoid where the neoplastic cells are enmeshed in a lymphoid cell rich stroma. The desmoplastic is probably the most difficult to be correctly recognized since it can simulate a fibrous pleuritis or a scarring process. Clue to malignancy are necrosis, cellular atypia, mitotic activity with atypical mitoses, stromal and especially adjacent soft tissue invasion. Abrupt transition from acellular to cellular areas featuring spindle cells is a useful clue to spindle cell mesothelioma, whereas an inflammatory cellular gradient with granulation tissue haphazardly admixed to fibrin and inflammatory cells speak in favor of pleuritis/inflammation. Another issue
would be to rule out either a sarcomatoid carcinoma with extension into the pleura or a sarcoma of soft tissue. The presence of an atypical spindle cell proliferation with stromal invasion and necrosis is usually a good sign of a sarcomatoid mesothelioma. In desmoplastic mesothelioma however the diagnosis can be very difficult when small biopsy fragments are available. In these cases, the pleural biopsy may show more collagenization and only a paucicellular proliferation which may not show marked cytologic atypia; in these cases, the diagnosis may not be apparent and one can only make such suggestion if the clinical and radiological findings are in keeping with such diagnosis.

**Immunohistochemical studies:** in the setting of a spindle cell mesothelioma whether the tumor is desmoplastic or not, the role of immunohistochemistry is relatively limited since most of the antibodies use in regular epithelial mesotheliomas have no practical use in sarcomatoid mesotheliomas. The use of broad-spectrum keratin is by far the most important of them. Calretinin may be positive, however synovial sarcoma – the most common intrathoracic spindle cell sarcoma – may positively react for calretinin. Keratin antibodies have some usefulness in demonstrating positivity of fibroblast-like tumor cells that infiltrate the chest wall soft tissue in cases of desmoplastic or spindle cell mesothelioma.

**Differential Diagnosis**

In cases in which there is no doubt about the neoplastic nature of the tumor, the most important differential diagnosis is with another spindle cell neoplasm of mesenchymal origin. In this case, the use of proper immunohistochemical studies and/or electron microscopy will lead to more appropriate interpretation. However proper clinicopathologic correlation are of great value. In doubtful cases, again, the pathologist is advised to render a provisional microscopic interpretation and if possible ask for more representative diagnostic material.

**C. Biphasic Mesotheliomas:** these neoplasms feature a mixture of epithelial and sarcomatoid areas. One important differential diagnosis with biphasic mesotheliomas is the fact that primary synovial sarcomas of the pleura have been described. However, in that setting the tumor is a pleural-based mass without diffuse involvement of the pleura. Once again, close clinical and radiological correlation is highly advised in that setting.

**Selected references**

Thymic carcinoma: update of current diagnostic criteria and histologic types

Giovanni Falconieri, M.D.

Thymic carcinoma is histologically defined as a primary thymic epithelial neoplasm showing overt cytologic features of malignancy with absence of the organotypical features of differentiation of the thymus. Since histology of thymic carcinoma is largely non-specific, the criteria for making this diagnosis should be, in the majority of instances, based on a combination of clinical and morphologic data. If a few cases are excepted, the diagnosis of thymic carcinoma cannot be established based solely on histopathologic examination. The role of histologic examination is mainly to confirm a diagnosis of malignancy (i.e., establish the presence of overt cytological atypia) and to specify the morphologic type of the lesion (i.e., squamous, basaloid, lymphoepithelioma-like, sarcomatoid, etc). It cannot be over-emphasized that there is nothing distinctive or pathognomonic about the histology of thymic carcinoma that can help establish a definitive diagnosis based solely on histologic examination of a biopsy or resected specimen. Accurate clinical and instrumental studies are needed to demonstrate the absence of an occult tumor elsewhere or to elicit a history of a remote primary. In a number of cases, the answer to this question may only be determined by means of a post-mortem examination. Important exceptions to this rule include cases in which obvious transitions with preexisting thymic epithelium or with well–differentiated areas displaying the conventional organotypical features of a preexisting thymoma are demonstrated or when dealing with some variants of thymic carcinoma whose features are so highly distinctive that origin from an alternate source other than the thymus would be highly unlikely, such as basaloid carcinoma of the thymus with cystic changes or carcinoma of the thymus with rhabdomyomatous cells.

Histopathologic types

Thymic carcinoma have a broad spectrum of microscopic features. A large number of histologic types of thymic carcinoma have been described, virtually identical to similar tumors arising in other organs. Phenotypically, thymic carcinomas range from that of low–grade, well–differentiated neoplasm, to a high–grade poorly–differentiated malignancy. A common feature of all thymic carcinomas is the fact that they lack any of the organotypical features of thymic differentiation (i.e., lobulation separated by broad fibrous bands originating from the capsule, perivascular spaces, areas of “medullary” differentiation, and a dual cell population composed of immature T-lymphocytes and thymic epithelial cells). When present, the lymphoid component in these tumors is made up of either mature T–cells or B–lymphocytes/plasma cells. The most common form of thymic carcinoma in Western patients is poorly–differentiated, non–keratinizing squamous cell carcinoma. Primary neuroendocrine carcinomas of the thymus are a special category that, although belonging in the same family of tumors, deserve special attention and will not be covered in this presentation.

Squamous cell carcinoma (SCC) of the thymus

Primary SCC of the thymus can manifest in three forms depending on their degrees of differentiation: well–differentiated (keratinizing) SCC; moderately–differentiated SCC, and poorly–differentiated (non–keratinizing) SCC which is by far the more prevalent in western countries. The latter often shows a distinctive syncytial growth pattern along to a heavy lymphoplasmacellular stromal infiltrate, and for that reason it was also called “lymphoepithelioma–like” carcinoma, resembling the homonymous lesion in nasopharynx. SCC is the most common type of thymic carcinoma. Most cases occur in middle aged adults with a slightly increased female ratio, presents as an anterior mediastinal mass, usually invades adjacent structures. Lymph node metastases are frequent.
- Well-differentiated, keratinizing SCC shows is fully comparable to its common counterpart in other organs. The cardinal microscopic features are usually present: nests or cords of large, atypical polygonal tumor cells showing a pavement-like, epidermoid arrangement, with large, vesicular nuclei with prominent eosinophilic nucleoli and mitoses. Thick cell membranes, intercellular bridges, foci of keratinization and squamous pearl formation are commonly identified. Focal necrosis may be present. SCC is widely invasive and tumor infiltration is associated with a desmoplastic stroma, along to an inflammatory infiltrate featuring neutrophils, eosinophils or lymphocytes. Cases exhibiting transitions with areas bearing the features of organotypical or atypical thymoma have been published. Rarely, association with multilocular thymic cysts has been described as well. The main differential diagnoses include a metastasis of SCC to mediastinal lymph nodes and atypical thymoma (WHO type B3 thymoma). Distinction of these tumors from atypical thymoma can be sometimes difficult. In fact, it is likely that many of the cases reported as well–differentiated SCC of the thymus in the literature actually correspond to atypical thymomas. The main distinguishing features between these two entities include the extensive, as opposed to focal nature of the keratinizing areas in SCC, and the demonstration of immature T-lymphocytes intimately admixed with the epithelial tumor cells in atypical thymoma. The possibility that well–differentiated SCC of the thymus arises as a result of tumor progression from atypical thymoma is supported by the frequent areas of transition observed between these two neoplasms and the overlap in histologic features that both can display. This could also explain the much more favorable prognosis of well–differentiated thymic carcinoma, which closely parallels that of atypical thymoma.

- Moderately-differentiated SCC is characterized by more pronounced cytologic atypia and less obvious evidence of squamous differentiation; the cells display a higher nuclear–to–cytoplasmic ratio, frequent mitoses and loss of clear-cut intercellular bridges. Keratinization is also focal and inconspicuous and keratin pearls are generally absent. Many of the tumor cells display single cell keratinization, with brightly eosinophilic cytoplasm surrounding small piknotic or degenerating nuclei. Areas of necrosis are more prominent than in the well–differentiated tumors and may be often confluent. Lymphovascular invasion is also commonly seen. Some tumors can show prominent peripheral palisading of tumor cells around small tumor islands. The most important differential diagnosis is with a metastasis from a SCC of the lung. This distinction is of clinical importance since primary squamous cell carcinoma of the thymus follows a better prognosis than a comparable primary squamous cell carcinoma of the lung with mediastinal lymph node involvement. In Shimosato et al series, cases in which the primary thymic tumors could be successfully excised and treated with postoperative radiation the patients were alive and well from 1–12 years after diagnosis. A careful clinical approach which includes bronchoscopic examination and detailed instrumental studies are recommended to demonstrate absence of bronchial compromise in such tumors and to rule out the possibility of massive mediastinal extension from a pulmonary primary lesion.

- Poorly-differentiated (non-keratinizing) SCC is characterized by sheets and islands of primitive-appearing round to oval tumor cells with large, vesicular nuclei, prominent, often centrally placed eosinophilic nucleoli, and scant rim of pale cytoplasm with indistinct cell borders. Foci of keratinization are rarely seen and intercellular bridges are always absent. The tumors often grow in a syncytial pattern separated by a dense lymphoid stroma closely reminiscent of the nasopharynx analogue lesion. Snover et al originally addressed the resemblance of these tumors with their nasopharyngeal counterpart; hence the “lymphoepithelioma-like carcinoma” appellation. The latter term, however, is somehow obsolete and is no longer used to refer to the nasopharyngeal tumors; it is therefore probably best abandoned. A striking and distinctive feature of these tumors is foci of central, comedo-like areas of necrosis within the tumor cell islands. On higher magnification, the tumor cells often display high mitotic activity (>10 mitoses x 10/HPF). The intervening stroma often displays a heavy lymphoplasmacellular infiltrate, however many tumors may be associated with a desmoplastic stroma devoid of lymphoid elements. The clue to the diagnosis lies in the identification
of the characteristic nuclear morphology featuring large vesicular nuclei with scant chromatin and centrally placed round eosinophilic nucleoli. Association with a preexisting thymoma has also been well-documented. In particular, a close relationship with spindle cell thymoma (WHO type A thymoma) has been observed raising thus the possibility that some cases may arise from transformation of spindle cell thymoma. Another association observed for these tumors is with Epstein-Barr viral (EBV) infection. A number of cases have been positive when tested for EBV by EBER in-situ hybridization or DNA analysis. The majority of the EBV-positive cases have occurred in children or young adults. The significance of this association is still unclear. In a large study of thymic carcinoma cases, including lymphoepithelioma-like carcinoma, no case of EBV-positive tumor was recognized. The author concluded that since most reported cases of EBV-associated tumors occur in young people, an age period when patients are most susceptible to EBV infection, the EBV may simply be an innocent bystander rather than having any pathogenetic implications. There is also an unusual case reported in the literature of lymphoepithelioma-like carcinoma of the thymus with focal neuroblastomatous differentiation demonstrated by ultrastructural examination. Poorly-differentiated nonkeratinizing squamous cell carcinoma is a highly aggressive tumor with a mean survival time of approximately 18 months.

As already mentioned, virtually all histotypes have been described in anecdotal cases usually in miniseries or compilation collected over very long period of time. An in-depth analysis may be found in current textbooks of surgical pathology and in recent reference papers. A summarized list of some of these microscopic entities is provided below, reminding again that clinicopathologic correlation is essential to make the correct diagnosis, i.e. rule out a metastatic tumor from another malignancy before considering the lesion as primary in thymus.

**Mucoepidermoid carcinoma (MEC).** It is characterized by the intimate admixture of squamous and mucinous components. MEC of thymus is virtually indistinguishable from its counterpart in the salivary glands and other organs. Although initially believed to represent low-grade carcinomas of the thymus, poorly-differentiated and widely invasive cases with a much more aggressive course have now been documented. Approximately 20 cases have been reported so far in the literature. Secondary cystic changes are relatively frequent and may be seen in almost half of the cases; they will manifest radiographically as multicystic masses on chest CT scans. Multiple multilocular structures of varying sizes filled with mucinous material are recognized macroscopically. Histologically the tumors can display either a well-differentiated, low-grade morphology or features of high-grade, poorly-differentiated MEC.

**Clear cell carcinoma (CCC) of the thymus.** CCC of thymus is a rare variant characterized by cells with abundant optically clear cytoplasm. Less than 15 cases have been reported so far in the literature. These tumors are usually regarded as a high-grade variant of thymic carcinoma: aggressive course is common, being characterized by massive local recurrence with infiltration of adjacent organs and distant metastases. A broad range of cytologic features was noted, from uniform clear cells with minimal atypia, to large, pleomorphic tumor cells with prominent nucleoli. In some cases, transitions of the clear cells with areas of conventional squamous cell carcinoma was documented. Cytoplasmic glycogen was often demonstrated.

**Basaloid carcinoma of the thymus (BCT).** BCT is another rare variant characterized by haphazardly arranged cords and islands of small, round to oval tumor cells characterized by peripheral palisading of nuclei recalling basal cell carcinomas of the skin. Cystic changes are common in BCT either as remnants of an acquired multilocular thymic cyst or secondary to cystic degeneration of the tumor itself. The overriding characteristic is the basaloid arrangement of nuclei at the periphery of the tumor cell islands. Mitotic figures are numerous and apoptotic cells can frequently be seen scattered throughout. Areas of necrosis are rare. Foci of squamous differentiation can also be occasionally identified.

**Carcinosarcoma.** This is a rare, biphasic thymic neoplasm characterized by clinical aggressiveness;
areas of epithelial differentiation (i.e., carcinoma) and areas composed of truly sarcomatous elements are observed microscopically. The epithelial component can be squamoid, adenosquamous, or poorly differentiated large cell or anaplastic. The term “sarcomatoid carcinoma” applied to these tumors in the past is a misnomer since they do not simply resemble a sarcoma, but actually contain true sarcomatous areas as part of their cellular constituents.

A differential diagnosis is with synovial sarcoma of the anterior mediastinum. Microscopically, synovial sarcoma is composed of a monotonous spindle cell proliferation admixed with scattered glandular elements. Unlike carcinosarcoma, both the spindle cell elements as well as the glandular component will show positivity for cytokeratin and EMA. In questionable cases, demonstration of the fusion product for the X:18 translocation will be of help in establishing the diagnosis of synovial sarcoma.

Papillary carcinoma, adenosquamous, desmoplastic and giant cell carcinoma and adenocarcinoma of the thymus, mucinous and non-mucinous subtypes, nut midline carcinoma with t(15;19) chromosomal translocation are very rare and only a few cases have been reported

Role of Immunohistochemistry

A series of markers have been studied in an attempt to separate thymic carcinoma from thymoma, and thymic carcinoma from a metastatic tumor, however little actual progress has been made in this regard and much of the available data has been contradictory or inconclusive. The common minimum denominator for these tumors is the expression of keratins in the tumor cells. Some studies have also shown increased expression of EMA in thymic carcinoma as opposed to thymoma. Initial studies of CD5, CD70 and CD117 seemed very promising in that it was proposed that detection of any of these markers may be used as positive proof to support the diagnosis of primary thymic carcinoma yet further studies have demonstrated that a significant proportion of thymic carcinoma are no–reactive with any of these antibodies. In addition, numerous extra–thymic neoplasms may be positive for these markers. Expression of c–kit (CD117) has attracted investigators as long as strong staining has been noted in squamous cell carcinoma of the thymus and this cell has been negative in the majority of the cases studied of thymoma and extra thymic squamous cell carcinomas. Yet, additional validating studies are needed.

References


Notes
CASE 1. Mass of the anterior mediastinum in a 37 year-old man complaining of shortness of breath.

Diagnosis: Basaloid carcinoma of the thymus

Selected references
CASE 2. Pleural tumor in a 39 year–old man presenting with chest pain, shortness of breath, general discomfort

Diagnosis: Synovial sarcoma of pleura

Selected references
CASE 3. Incidental pleural mass discovered in a 78 year-old man

Diagnosis: Solitary fibrous tumor

Selected references
CASE 4. Mass of posterior mediastinum growing within the costo–vertebral angle in a 32 year–old man

Diagnosis: Malignant (low-grade) peripheral nerve sheath tumor

Selected references
CASE 5. Mass biopsy fragments in a 69 year-old man with diffuse pleural thickening and clinical diagnosis of mesothelioma

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Diagnosis: Metastatic melanoma

Selected references:

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CURRENT APPOINTMENT(s)
MD, Instituto Português de Oncologia – Porto Jan – 2004

RELEVANT QUALIFICATIONS
Oporto University, MD, 1994
Hospital Santo António, MD, 1996
Instituto Português de Oncologia – Porto, MD, 2002
Ludwig Institute for Cancer Research / Sant Luc Hospital, MD, 2002
Hospital S. João, MD, 2003
Instituto Português de Oncologia – Oporto, Medical Oncology, 2004

PREVIOUS APPOINTMENTS
Sant Luc Hospital – Brussels, from Oct 2001 to Aug 2002
Hospital de S. João – Oporto, from Sep 2002 to Dec 2003
Instituto Português de Oncologia – Oporto, from Jan 2004 to Present date

RESEARCH EXPERIENCE in relevant Clinical Therapeutic Trials
Instituto Português de Oncologia – Oporto, from Jan – 2004 to Present date
Immunotherapy on the treatment of advanced melanoma

The immune system has the potential for the destruction of tumors and the long term memory can prevent cancer recurrence. It is known that malignant melanoma is an immunogenic tumor for decades. Tumor specificity of the immune response is based in the recognition of tumor antigens. But in a majority of cancers malignant progression is accompanied by immune suppression that interferes with an effective antitumor destruction. One of the negative regulator mechanism is the activation of the cytotoxic T-Lymphocyte antigen–4 (CTL–4), a negative regulator of T–cell activation. In consequence, infiltrating T–cells fail to be sufficiently activated to result in tumor destruction.

Ipilimumab is a novel agent that belongs to a class of immunomodulatory agents which alters the balance of the immune system. It is a monoclonal antibody targeting the CTLA–4, so increasing the T lymphocytes proliferation and tumor destruction. Phase III studies with ipilimumab for the first time in the history of metastatic melanoma showed significantly increase in progression free survival and overall survival.