PATHOLOGICA

Journal of the Italian Society of Anatomic Pathology and Diagnostic Cytopathology, Italian Division of the International Academy of Pathology

ORIGINAL ARTICLES
169 A fluorescence in situ hybridization (FISH) procedure to assist in differentiating benign from malignant melanocytic lesions

175 True thymic hyperplasia versus follicular thymic hyperplasia: a retrospective analysis of 13 cases
   M. Mlika, A. Ayadi-Kaddour, A. Marghli, O. Ismail, T. Kilani, F. El Mezni

CASE REPORTS
180 Bronchial fibrolipomatous hamartoma associated with peculiar unusual bronchial lesions of the peripheral lung

183 Giant genital cavernous haemangioma: case description and surgical management
   M.C. Sighinolfi, L. Reggiani Bonetti, S. De Stefani, M. Pinelli, S. Micali, A. Maiorana, L. Schirosi, G. Sartori, C. De Gaetani, G. Bianchi

186 Pulmonary lymphangioliomyomatosis in tuberous sclerosis. A case report
   N. De Rosa

PROCEEDINGS
191 5th National Symposium on Cytopathology
   Turin, November 8-10, 2009

SIAPEC-IAP
Società Italiana di Anatomia Patologica e Citopatologia Diagnostica, Divisione Italiana della International Academy of Pathology
A fluorescence in situ hybridization (FISH) procedure to assist in differentiating benign from malignant melanocytic lesions


Pathology and Cytopathology Unit, Casa di Cura San Pio X, Milan, Italy; *Cytogenetic Laboratory, Operative Unit of Clinical Investigations, IRCCS, Humanitas Clinical Institute, Rozzano, Milan, Italy; **Cancer Genetics Service, Casa di Cura San Pio X, Milan, Italy; ***Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

Key words
Melanocytic lesions • Melanoma • Differential diagnosis • Molecular genetics • In situ hybridization

Summary
Objective. Misdiagnosis of melanocytic lesions can result in unnecessary psychological distress to patients, under- or overtreatment, inaccurate prognosis and improper follow-up and family member surveillance. It is well recognized that, despite many attempts to 1) put forth a set of histologic criteria that can accurately and reproducibly be used to diagnose melanocytic lesions, and 2) identify reliable markers of malignancy as an adjunct to routine histopathology, misdiagnoses still occur in a significant number of cases.

Method. A multi-color FISH probe mixture has been devised to assist pathologists in differential diagnosis of difficult melanocytic lesions. The mixture includes a centromeric probe for chromosome 6 and unique sequence probes for three other chromosomal regions that have most frequently shown amplifications or deletions in melanoma. We have carried out a preliminary evaluation of this new probe set in 25 cases of benign and malignant pigmented lesions.

Results. The tool reliably identified all nevi and ordinary melanomas, and only failed to identify a pigmented epithelioid melanocytoma and two malignant lesions that, by morphology and behavior, have distinct features from common invasive melanomas, i.e., a desmoplastic melanoma and a nevoid melanoma. Considering this, 100% specificity and 75% sensitivity was achieved.

Conclusion. The FISH tool used in this study was able to separate accurately benign nevi from ordinary melanoma. Failure to identify uncommon melanocytic lesions adds to its advantage and calls for further studies to unveil the molecular profile of these rare entities.

Introduction
Although there has been great strides in understanding and diagnosing pigmented lesions in the last 30 years, there still remain melanocytic proliferations that defy definitive classification and prediction of clinical behavior. Because significant misdiagnoses occur as much as half of these difficult cases, markers for malignancy have been actively searched for as an adjunct to routine histopathology. Despite several attempts to differentiate benign from malignant pigmented lesions through different techniques,1-8, no tool has emerged as a reliable aid for general use with all melanocytic lesions. Although these challenging cases can be approached by asking for a second opinion from an expert pathologist, lack of agreement even among expert dermatopathologists is not uncommon.9-11.

The molecular biology revolution and the advent of genomic technologies have facilitated rapid advances in our understanding of the molecular details of cell and tissue function. Our improved understanding of the molecular basis of various pathologies has also unveiled associations between genetic variations and biological outcomes, whose potential usefulness is being tested. In the field of pigmented lesions, an active search is undergoing to look for molecular differences between benign and malignant pigmented lesions, as well as differences among various types of malignant melanoma. To accomplish this task, comparative genomic hybridization (CGH) is often used, which allows for an assessment of the entire genome to detect abnormalities in DNA copy numbers and identifies malignant melanomas by their prominent chromosomal instability. This change is manifested by the presence of

Acknowledgement
The probe set was supplied by Abbott Molecular (Des Plaines, Illinois, U.S.A.)

Correspondence
Dr Claudio Clemente, Servizio di Anatomia Patologica e Citopatologia, Casa di Cura “S. Pio X”, via F. Nava 31, 20159 Milano, Italy.
gains or losses in chromosomes or in portions thereof in about 95% of cases. Melanocytic nevi, on the other hand, are genomically stable and CGH represents a good tool that allows distinguishing nevi from melanoma based upon the absence of chromosomal gains or losses.\textsuperscript{12-16} Fluorescence in situ hybridization (FISH) represents a more targeted approach than CGH, since it has the ability to identify locus-specific alterations. Various studies have demonstrated its usefulness in identifying chromosomal alterations in melanomas.\textsuperscript{17,18}

Recently, a multi-color FISH probe mixture has been devised by Abbott Molecular (Des Plaines, Illinois, U.S.A.) to assist pathologists in differential diagnosis of difficult melanocytic lesions. The probe mixture includes a centromeric probe for chromosome 6 and unique sequence probes for the \textit{RREB1} gene (located at 6p25), \textit{MYB} gene (located at 6q23-q23), and \textit{CCND1} gene (located at 11q13). The centromeric probe (CEP6) was included as a control for the ploidy level of chromosome 6, while the other three were chosen because their respective chromosomal regions have most frequently shown amplifications or deletions in melanoma. \textit{RREB1} gene (ras responsive element binding protein 1) encodes a transcription factor that binds specifically to the RAS-responsive elements (RRE) of gene promoters.\textsuperscript{19} \textit{MYB} is an oncogene that also encodes a transcription factor, and rearrangement of chromosome 6 has long been demonstrated in melanoma.\textsuperscript{20} \textit{CCND1} gene encodes for cyclin D1, a major regulator of the cell cycle transition from G1 phase to S phase\textsuperscript{21} and an increase in its copy number has been observed in a subset of melanomas.\textsuperscript{15} Along these lines, this 4-probe set was selected to ensure optimal sensitivity and specificity in the detection of melanoma.\textsuperscript{22-24}

The present paper reports preliminary results from ongoing work to test this novel tool in benign and malignant pigmented lesions.

Material and methods

Twenty-five lesions from 24 patients were collected. Archival paraffin blocks were used, corresponding to benign and malignant pigmented lesions reported by one of the authors (CC). Two of the specimens were from the same patient (Tab. I). The cases were selected from among 174 melanocytic lesions (corresponding to 152 patients) consecutively reported (CC) at two institutions between July 10\textsuperscript{th} and October 4\textsuperscript{th}, 2007. The selection criteria adopted included unequivocal diagnosis of benign or malignant melanocytic proliferation, sufficient amount of tissue representative of the lesion, and good preservation of tissue morphology as judged by the H&E diagnostic slides. Three consecutive 4-\textmu m-thick sections were cut from each of the retrieved paraffin blocks and mounted on glass slides (SuperFrost\textsuperscript{®}Plus, Mentzel, Braunschweig, Germany): the first and the last profiles were stained with H&E and were used to accurately identify the area(s) containing the cells of interest, while the middle section was used for FISH analysis.

Samples for FISH analysis were treated following the manufacturer’s protocol with minor modifications. Slides were incubated overnight at 50°C, deparaffinized by washing twice for 5 minutes in Microclearing (DiaPath, Martinengo, BG, Italy) solution and dehydrated twice in 100% ethanol for 1 minute. They were then pretreated in 1X SSC pH 6.3 at 80°C for 35 minutes and rinsed 3 minutes in purified water. Protease digestion was carried out for 15 minutes at 37°C in a protease buffer II solution composed of 250 mg pepsin powder (Sigma, catalogue number P6887, 3 200 - 4 500 units/mg protein) in 62.5 ml 0.2 N hydrochloric acid (Abbott Laboratories, Abbott Park, IL, USA). Slides were then washed in purified water for 3 minutes and dehydrated for one minute in 70%, 85% and 100% ethanol. Following air-drying, sections were covered with 10 \textmu l of the 4-colour probe Vysis LSI\textsuperscript{(R)RREB1} (Spectrum Red) / LSI\textsuperscript{(R)MYB} (Spectrum Gold) / LSI\textsuperscript{(R)CCND1} (Spectrum Green) and CEP\textsuperscript{6} (Spectrum Aqua) (Abbott Laboratories, Abbott Park, IL, USA). A 22x22 mm coverslip was then applied and its margins sealed with rubber cement. Co-denaturation (73°C for 5 minutes) and hybridization (37°C overnight) processes were carried out in a HYBrite oven (Abbott Molecular, see above). After gentle removal of the coverslip, slides were soaked twice in 2X SSC/0.3% NP-40, respectively for 5 minutes at room temperature and for 2 minutes at 73°C, and air-dried in darkness. Ten \textmu l of DAPI counterstain (DAPI I, Abbott Molecular) were then added onto the sections, and a coverslip applied. A period of at least 1 hour at -20°C was allowed before microscopic observation was started using an Olympus BX 61 fluorescent microscope (Olympus Italia Srl, Milan, Italy) equipped with a Chroma filter set (DAPI/spectrum red/spectrum green/spectrum aqua/spectrum orange) (Rockingham, VT, USA). Scoring was restricted to cells from the areas previously identified on matched H&E sections and was carried out independently by two FISH-experienced cytogeneticists (DB and AV), without prior knowledge of the diagnosis. The manufacturer suggested considering signals from 30 non-overlapping nuclei, selected from three different areas of the lesion. Whenever feasible, however, scoring was extended to 60 non-overlapping intact nuclei. In a normal cell, the expected signals for each probe should be two. As indicated by the manufacturer, a specimen was considered FISH-positive if the average signals per nucleus of \textit{CCND1} or \textit{MYB} was 2.5 or if the percent loss of \textit{MYB} against CEP6 (percentage of cells where the number of signals for \textit{MYB} was less than those for CEP6) was 31% or if the percentage of nuclei with \textit{RREB1} signals greater or lesser than 2 was 63%. A specimen was scored as FISH-negative if none of the above criteria were met.

Results

Twenty-two specimens were evaluated following elimination of three samples because of technical reasons (Tab. II). Case 6 was not evaluable because of the abun-
dance of overlapping nuclei. Case number 15 fell out during the hybridization procedure, and case 19 did not display any hybridization signal in two separate experiments with control slides.

Six out of eight invasive melanomas were scored as FISH-positive (Fig. 1), while all 12 nevi were scored as negative, including the one associated with melanoma in case 23 (Fig. 2). The only melanoma in situ was also scored as negative, as well as the pigmented epithelioid melanocytoma (PEM; Tab. II).

None of the specimens displayed a positive score for all four probes. Among invasive melanomas one lesion was positive for three probes, two lesions were positive for two probes, and three lesions were positive for one probe each. In most cases, positivity ensued from gain of CCND1 and/or loss of MYB (Tab. II).

Discussion

In agreement with previous findings, our results demonstrate that invasive melanomas can be reliably distinguished from an array of benign and indolent melanocytic lesions by FISH analysis based on a probe set.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Patient sex</th>
<th>Patient age (yrs)</th>
<th>Lesion site</th>
<th>Histopathological diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>M</td>
<td>71</td>
<td>back</td>
<td>Melanoma, lymph node metastasis; TIL absent</td>
</tr>
<tr>
<td>02</td>
<td>M</td>
<td>59</td>
<td>thigh</td>
<td>Superficial spreading melanoma, Clark III, Breslow 1.66 mm, &gt;6 mitoses per mm², TIL non-brisk</td>
</tr>
<tr>
<td>03</td>
<td>M</td>
<td>11</td>
<td>back</td>
<td>Dermal nevus, congenital-like, verrucous</td>
</tr>
<tr>
<td>04</td>
<td>M</td>
<td>30</td>
<td>neck</td>
<td>Dermal nevus, with involutive sclerosis and features of pseudoinvasion</td>
</tr>
<tr>
<td>05</td>
<td>F</td>
<td>36</td>
<td>breast</td>
<td>Reed’s pigmented spindle cell nevus, with regression and focal atypia</td>
</tr>
<tr>
<td>06</td>
<td>M</td>
<td>52</td>
<td>back</td>
<td>Melanoma, embolic lymph node metastasis (sentinel procedure)</td>
</tr>
<tr>
<td>07</td>
<td>F</td>
<td>65</td>
<td>arm</td>
<td>Superficial spreading melanoma, Clark IV, Breslow 1.2 mm, 16 mitoses per mm², TIL brisk</td>
</tr>
<tr>
<td>08</td>
<td>F</td>
<td>73</td>
<td>abdomen</td>
<td>Dysplastic nevus</td>
</tr>
<tr>
<td>09</td>
<td>M</td>
<td>64</td>
<td>back</td>
<td>Small cell nodular melanoma, Clark IV, Breslow 6 mm, &gt;6 mitoses per mm², TIL absent</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>34</td>
<td>thorax</td>
<td>Compound nevus, with sclerosis and plaque-like Spitz features</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>39</td>
<td>shoulder</td>
<td>Desmoplastic melanoma, Clark IV, Breslow 1.5 mm, 16 mitoses per mm², TIL non-brisk</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>17</td>
<td>leg</td>
<td>Pigmented epithelioid melanocytoma</td>
</tr>
<tr>
<td>13 *</td>
<td>F</td>
<td>33</td>
<td>back</td>
<td>Dysplastic nevus</td>
</tr>
<tr>
<td>14 *</td>
<td>F</td>
<td>33</td>
<td>abdomen</td>
<td>Melanoma in situ</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>1</td>
<td>umbilicus</td>
<td>Proliferative dermal nodule within a compound nevus; presence of some mitoses</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>53</td>
<td>back</td>
<td>Desmoplastic blue nevus</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>46</td>
<td>hip</td>
<td>Superficial spreading melanoma, Clark IV, Breslow 1.4 mm, 16 mitoses per mm², TIL absent</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>44</td>
<td>foot</td>
<td>Acral compound nevus</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>34</td>
<td>thigh</td>
<td>Superficial spreading melanoma in horizontal growth phase, Clark II, Breslow 0.4 mm, 1 mitosis per mm²</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>34</td>
<td>forearm</td>
<td>Reed’s pigmented spindle cell nevus</td>
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<tr>
<td>21</td>
<td>M</td>
<td>63</td>
<td>ankle</td>
<td>Compound nevus, with atypical intraepithelial epithelioid hyperplasia</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>52</td>
<td>foreskin</td>
<td>Dermal nevus</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>62</td>
<td>abdomen</td>
<td>Superficial spreading melanoma, Clark III, Breslow 0.62 mm, &lt;1 mitoses per mm², TIL brisk, with associated compound nevus</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>68</td>
<td>hip</td>
<td>Nodular melanoma, Clark IV, Breslow 2.2 mm, &gt;6 mitoses per mm², TIL absent</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>28</td>
<td>back</td>
<td>Compound nevus</td>
</tr>
</tbody>
</table>

LEGEND: * the two lesions are from the same patient; the level of invasion was reported as defined by Clark 37; the lesion thickness was measured using Breslow criteria 38; mitotic count was expressed as the mitotic count per square millimeter and was given as < 1 figure, 1-6 figures, and > 6 figures 39; TIL = tumor-infiltrating leukocytes, expressed as absent, non-brisk, and brisk 40.
targeting RREB1, MYB, and CCND1 genes. Interestingly, the melanoma in situ and the PEM were negative. The melanoma in situ was of the acral lentiginous type. This represents a distinct type of melanoma characterized by an indolent clinical course and a long dormancy period before transforming into a more aggressive invasive melanoma. As far as the PEM is concerned, recent data suggests that this tumor is different from melanoma and classical nevi, occurring both sporadically and in the context of the Carney complex. PEM is also an entity that in general shows an indolent clinical course, even with frequent lymph node involvement. Two out of the eight invasive melanomas analyzed were also scored as negative: one was a desmoplastic

<table>
<thead>
<tr>
<th>Case no.*</th>
<th>Lesion type</th>
<th>FISH results</th>
<th>% of nuclei with RREB1 signals ≠2 §</th>
<th>Average CCND1 signals per nucleus §§</th>
<th>Average MYB signals per nucleus §§§</th>
<th>Percent loss of MYB against CEP6 §§§§</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Melanoma, primary</td>
<td>+</td>
<td>75.00</td>
<td>3.40</td>
<td>2.01</td>
<td>64.16</td>
</tr>
<tr>
<td>1</td>
<td>Melanoma, metastatic</td>
<td>+</td>
<td>61.70</td>
<td>2.70</td>
<td>3.50</td>
<td>5.80</td>
</tr>
<tr>
<td>23</td>
<td>Melanoma, primary, with associated nevus **</td>
<td>+</td>
<td>57.60</td>
<td>2.63</td>
<td>1.61</td>
<td>51.60</td>
</tr>
<tr>
<td>2</td>
<td>Melanoma, primary</td>
<td>+</td>
<td>59.20</td>
<td>2.33</td>
<td>2.46</td>
<td>31.70</td>
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<tr>
<td>7</td>
<td>Melanoma, primary</td>
<td>+</td>
<td>48.00</td>
<td>2.54</td>
<td>2.45</td>
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<td>9</td>
<td>Melanoma, primary</td>
<td>+</td>
<td>45.80</td>
<td>1.80</td>
<td>1.73</td>
<td>36.70</td>
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<td>-</td>
<td>29.20</td>
<td>2.07</td>
<td>2.01</td>
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<tr>
<td>17</td>
<td>Melanoma, primary</td>
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<td>31.60</td>
<td>1.79</td>
<td>1.88</td>
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<td>Pigmented epithelioid melanocytoma</td>
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<td>13.30</td>
<td>1.89</td>
<td>1.99</td>
<td>11.60</td>
</tr>
<tr>
<td>14</td>
<td>Melanoma in situ</td>
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<td>1.67</td>
<td>1.93</td>
<td>1.85</td>
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<td>Nevus</td>
<td>-</td>
<td>25.80</td>
<td>1.92</td>
<td>1.63</td>
<td>16.60</td>
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<td>3</td>
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<td>-</td>
<td>23.30</td>
<td>1.88</td>
<td>1.60</td>
<td>23.30</td>
</tr>
<tr>
<td>25</td>
<td>Nevus</td>
<td>-</td>
<td>15.80</td>
<td>2.03</td>
<td>1.96</td>
<td>11.60</td>
</tr>
<tr>
<td>8</td>
<td>Nevus</td>
<td>-</td>
<td>18.00</td>
<td>1.85</td>
<td>1.65</td>
<td>19.00</td>
</tr>
<tr>
<td>5</td>
<td>Nevus</td>
<td>-</td>
<td>15.00</td>
<td>1.83</td>
<td>2.00</td>
<td>12.00</td>
</tr>
<tr>
<td>20</td>
<td>Nevus</td>
<td>-</td>
<td>13.30</td>
<td>1.86</td>
<td>1.94</td>
<td>7.50</td>
</tr>
<tr>
<td>16</td>
<td>Nevus</td>
<td>-</td>
<td>9.17</td>
<td>2.18</td>
<td>1.94</td>
<td>8.30</td>
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<tr>
<td>21</td>
<td>Nevus</td>
<td>-</td>
<td>9.16</td>
<td>1.93</td>
<td>1.85</td>
<td>7.14</td>
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<tr>
<td>22</td>
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<td>-</td>
<td>7.50</td>
<td>1.89</td>
<td>1.73</td>
<td>13.30</td>
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<tr>
<td>10</td>
<td>Nevus</td>
<td>-</td>
<td>5.83</td>
<td>1.94</td>
<td>1.91</td>
<td>10.00</td>
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<tr>
<td>4</td>
<td>Nevus</td>
<td>-</td>
<td>3.33</td>
<td>1.83</td>
<td>1.91</td>
<td>3.33</td>
</tr>
<tr>
<td>13</td>
<td>Nevus</td>
<td>-</td>
<td>2.50</td>
<td>1.91</td>
<td>1.87</td>
<td>15.00</td>
</tr>
</tbody>
</table>

**LEGEND:** § positive if >63%; §§ positive if >2.5; §§§ positive if >31%; * see Table I ** melanoma was +, nevus was negative. Positive scores are emphasized in bold.

Fig. 1. Representative FISH appearance of case 12 (pigmented epithelioid melanocytoma): two dots for each marker are present in all cell nuclei analyzed in the three areas selected, thus reflecting the widespread occurrence of two copies of the respective gene/chromosome. The red color indicates the RREB1 gene, the gold color MYB, and the green color CCND1, while the centromeric region of chromosome 6 appears in light blue.

Fig. 2. Representative FISH analysis of case 23 (primary invasive melanoma): more than two dots for each marker are frequently observed in nuclei from tumor cells. For the color legend, refer to Fig. 1.
melanoma, while the other had features of nevoid melanoma. Desmoplastic melanomas and nevoid melanomas are rare histopathologic variants.28-30, and a recent meta-analysis on 17 studies encompassing a total of 856 patients with desmoplastic melanoma showed that the incidence of nodal metastasis is lower in these patients than in patients with other forms of cutaneous melanoma.31 Moreover, in patients with this rare variant local recurrence rates are low and wide local excision with careful attention to appropriate margins produces excellent local control,32, a result that seems to be reinforced by adjuvant radiotherapy.33 Nevoid melanoma, according to some studies, does not appear to have a better prognosis than ordinary melanoma.34-36 However some authors have suggested that nevoid melanoma, when the criteria are strictly applied, may have a more indolent course than ordinary melanomas.34-36 Despite these studies, the rarity of nevoid melanomas (about 3% of all cutaneous melanomas) and their morphologic features reminiscent of a benign melanocytic nevus call for further studies to unravel its molecular profile.

In conclusion, the screening tool used in this study was able to accurately separate benign nevi from ordinary malignant melanoma. It reliably identified all nevi and only failed to identify two malignant lesions that, by morphology and behavior, have distinct features from common invasive melanomas. Although our scoring algorithm was somewhat different from that used by two other groups, we achieved 100% specificity and 75% sensitivity.32-34 Obviously, a wider array of melanocytic lesions with follow up data will need to be analyzed, including borderline lesions.35-36 The latter will demonstrate the real utility of this tool in assessing these morphologically uncharacteristic lesions.

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True thymic hyperplasia versus follicular thymic hyperplasia: a retrospective analysis of 13 cases

M. MLIKA, A. AYADI-KADDOUR, A. MARGHLI*, O. ISMAIL, T. KILANI*, F. EL MEZNI
Department of Pathology, Department of Thoracic Surgery; Abderrahmen Mami Hospital, 2080 Ariana, Tunisia

Key words
Thymic hyperplasia • Thymectomy • Follicular thymic hyperplasia • Myasthenia gravis • Cutaneous leishmaniasis

Introduction
Thymic enlargement is a rare phenomenon that can be challenging.

Materials and methods
We present a retrospective study over a 13-year period (from 1996 to 2008) of 13 cases of thymic hyperplasia that included 4 cases of true thymic hyperplasia (TTH) and 9 cases of follicular thymic hyperplasia (FTH).

Objectives
To describe the pathogenesis and different associations of these 2 entities; to emphasize the difficulties in accurate diagnosis without histological examination and to report an unusual association between TTH and cutaneous leishmaniasis.

Results
There were 9 women and 4 men. All patients with FTH had a past medical history of myasthenia gravis (MG). In the group of TTH, 2 patients received chemotherapy and one patient had been administered amphotericin B for a cutaneous leishmaniasis. Radiologic findings showed a mediastinal mass in 7 cases. Thymectomy was performed in all patients with FTH and in one patient with TTH and suspect malignant disease. We observed no complications in any case.

Conclusion
The two main types of thymic hyperplasia have different pathogenic characteristics and diverse treatment modalities.

Introduction
Two major types of thymic hyperplasia have been described in the literature: true thymic hyperplasia and follicular thymic hyperplasia. In young infants and neonates, a third type of massive thymic hyperplasia has also been described. The pathogenesis of thymic enlargement is still debated and seems to be different in the two main types of thymic enlargement inducing different therapeutic modalities.

Material and methods
Over a 13-year period (from 1996 to 2008), 13 cases of thymic hyperplasia were diagnosed at the Department of Pathology at Abderrahmen Mami Hospital. Clinical records, histological reports and microscopic slides were available in all cases and were retrospectively reviewed. Our study contained 2 groups: four patients with true thymic hyperplasia (TTH) and nine with follicular thymic hyperplasia (FTH). All patients underwent thorough clinical examination, standard chest radiographs and CT scans. Diagnosis of thymic hyperplasia was made on surgical biopsies in 3 cases and on thymectomy specimens in 10 cases. All samples were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin.

Results

Characteristics of the studied population
In the group of patients with TTH, there were 2 women and 2 men (sex ratio M/F = 1) ranging in age from 6 to 50 years (mean age 22 years). Three patients were asymptomatic. The first was treated for a thyroid carcinoma that was discovered 10 years before the appearance of the mediastinal mass. She underwent treatment with radioactive iodine, and a CT-scan was performed because of an increase of tumour markers suggesting eventual recurrence. The second patient was treated for an extra-skeletal Ewing’s sarcoma. He received chemotherapy and presented a mediastinal mass after one year. The third patient was treated for a cutaneous leishmaniasis and presented an incidental mediastinal mass. This patient was put on amphotericin B. The fourth patient presented with dyspnea and shortness of breath secondary to a viral syndrome. The symptoms decreased after steroid therapy.

In the group of patients with FTH, there were 7 women and 2 men (sex ratio M/F = 3.5) ranging in age from 16
to 40 years (mean age 24 years). All patients had a past medical history of seropositive myasthenia gravis (MG) that was severe and resistant to medical treatment in 8 cases. A chest-x-ray was made in one case in order to assess the etiology of the MG. Patient characteristics are shown in Table I.

**Diagnostic techniques**

Chest-x-ray showed a mediastinal enlargement in one case of TTH (Fig. 1) and a mediastinal mass in another. It was normal in the remaining cases. CT demonstrated an anterior mediastinal mass in 6 cases (4 TTH, 2 FTH), an enhanced triangular shaped thymus by contrast CT in one patient with FTH (Fig. 2) and was normal in the other cases.
**Histological Findings**

Histological study showed different features. TTH was characterized by thymus with increased weight and normal architecture, and the cortex and the medulla were conserved. FTH was characterized by a normal weight thymus with abnormal architecture. Many follicles were found within the cortex and the medulla (Figs. 3, 4).

**Discussion**

Thymic hyperplasia is described in association with other diseases that differ according to histological type. FTH has been reported in several autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, Hashimoto thyroiditis, Graves’ disease, autoimmune hemolytic anemia, Behcet’s disease and MG. It has also been described after resolution of hypercortisolism in Cushing’s syndrome, after resection of bronchial carcinoid tumours, corticotropic adenomas, following the administration of mitotane or ketoconazole and in association with hyperthyroidism and HIV infection.

Other incidental associations have also been reported with multifocular thymic cyst or Pena-Shokeir type I syndrome. The pathogenesis of all these associations is still unclear, and only some associations have been documented in the literature.

The association of MG with FTH has been frequently reported in the literature. In this case, patients with thymic enlargement are younger at the onset of symptoms and predominantly female. In this group, the HLA association (mainly HLA-B8 ad HLA-DR3) has been noticed in the absence of a true cause-effect relationship. Some authors have suggested the direct involvement of the T-cell system in the initiation or the regulation of AChR autoimmunity. In an immunohistochemical study, Roxanis and coworkers reported that the striking histological feature consists in bands of medullary thymic epithelial cells. It was reported that together with the expression of AChR subunits, these cells could be involved in the autoimmune response. In fact, it was shown that these cells were intermingled with abundant deposits of extracellular matrix proteins and expressed high levels of some integrins, stimulating their focal expansion and migration via fenestrations of the basement membrane into the extraparenchymal lymphoid component. As some apparently express AChR epitopes, they could potentially sensitize auto-aggressive T cells and initiate an autoimmune process. FTH in Graves’ disease seems to be more likely an association rather than a true cause. Marakami et al. demonstrated the presence of thyrotropin receptor transcripts and protein in non-neoplastic thymic tissue using Northern and Western blot analysis.

In contrast to FTH, TTH is considered as a rebound phenomenon and is common following recovery from an acute debilitating illness or chemotherapeutic treatment of a malignant disease (usually lymphoma, nephroblastoma, testicular teratoma, leukemia, Ewing’s sarcoma, osteosarcoma, ovarian or testicular tumour), in association with thyrotoxicosis, chronic adrenal insufficiency, in patients with MG. The association of TTH with Hodgkin lymphoma or chemotherapy is still unexplained. As the thymus is a T-cell organ, and Hodgkin’s lymphoma is characterized by T-cell dysfunction, some authors hypothesized relationship between chemotherapy and T-cell maturation. Enlargement of the thymus may represent a rebound response from the thymus to the treatment.
immunosuppressive effects of neoplasia and chemotherapy. Patient #8 presented an unusual association between cutaneous leishmaniasis and TTH. Cuna et al. demonstrated that the amphotericin B interferes with the T(h)1-cell response, which may be the cause of thymic enlargement in our case.11

The clinical presentation of thymic hyperplasia does not differ between TTH and FTH. In adults, a review of the literature showed that the most common presentation of thymic enlargement consists in signs of respiratory distress.12 Clinical presentation in children with thymic enlargement is non-specific and variable.1 Our study includes one child who presented signs of respiratory distress following a viral syndrome. This may be due to a bias in patient selection as our hospital selects only adults. Many radiological aspects have been studied using FDG-PET, CT, gallium-67 scintigraphy and chemical shift MR-imaging, although these results have not led to any consensus features.13 Radiological findings generally consist in enlargement of the anterior mediastinum. On CT, an enlarged gland due to hyperplasia often shows a triangular shape, whereas an infiltrated thymus has a quadrilateral shape and lobulated borders.15 These findings are not however diagnostic. In a retrospective study of 4 cases of TTH following chemotherapy in children, Aribal et al. reported a lobulated mass in one case corresponding histologically to a thymic hyperplasia.16 In our study, the CT scan showed a lobulated mass in the first patient and an abnormal enhanced thymus in case #7 so that, the radiological findings are not diagnostic. In adults, the presence of a mediastinal mass may be suggestive of thymoma, lymphoma, a germ cell tumour, mesenchymal tumour, thyroid or parathyroid masses or metastatic tumours. In neonates and young infants, differential diagnoses include teratoma, lymphangioma and lipoma, in addition to a physiological reaction to breast feeding and thymic disorders.5 Some authors have proposed cytopathological analysis after fine-needle aspiration biopsy guided by ultra-sound or CT-scan, which is simpler and less invasive, but it may also lead to misdiagnosis.16 Others have reported the importance of thymic vein catheterization to explore the ACTH gradient in order to make diagnosis in case of thymic enlargement secondary to a hypercortisolism resolution.9

Diagnosis is based mainly on histological findings. FTH is characterized by the presence of hyperplasic lymph follicles in the thymus independent of the gland’s size, whereas TTH is described as a normal thymic architecture consisting of cortex and medulla with Hassall’s corpuscles and an increase in thymic weight for a particular sex and age group.18

The treatment of thymic enlargement is still debated. The majority of authors use therapeutic modalities that are based on the different pathogenesis of these 2 main types. Because of the apparent implication of the thymus in the pathogenesis of myasthenia gravis, thymectomy seems to be the best choice of treatment. Different surgical approaches have been discussed in the literature including sternotomy and cervical incision. Cervical incision seems to have a lower morbidity but a poorer visualization of the mediastinum. Some patients may show only partial improvement of their clinical status. All our patients were treated by thymectomy through sternotomy, whereas one patient (#6) did not shown any improvement. In contrast to MG, the removal of the thymus in patients with Graves’ disease does not reverse hyperthyroidism. When an anterior mediastinal mass is associated with hyperthyroidism without radiologic signs of malignancy, Adriane et al. proposed close radiologic follow up.9 An eventual decrease in the mass’s size supports a diagnosis of thymic hyperplasia. The appearance of thymic hyperplasia after the resolution of hypercortisolism has been attributed by some authors to the variable resistance of the lymphoid tissue which is inferior to that of the stroma and epithelial tissues. Treatment consists in the use of glucocorticoids, which induce thymic involution.

The treatment of TTH remains debated. Some authors use oral prednisone (60 mg/m²/day for 7-10 days) and recommend an open biopsy when they fail to decrease the size of the mass.5 In our study, the patient #5 presented a decrease in thymic mass after steroid administration and an increase of the mass when the steroids were stopped, and thus a surgical biopsy was kaken. Clinical studies indicate that thymectomy in children less than 1 year of age results in decreased counts of peripheral blood T cell subsets. Therefore, thymectomy should be avoided whenever possible.1

Conclusion

Thymic enlargement is a rare phenomenon which may create a dilemma regarding differential diagnosis between a thymic hyperplasia and a mediastinal tumour. Diagnosis must be based on histological findings, and the treatment modalities depend on the subtype of hyperplasia and the different clinical associations.

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Bronchial fibrolipomatous hamartoma associated with peculiar unusual bronchial lesions of the peripheral lung

Department of Human Pathology and Oncology, Section of Anatomic Pathology, University of Siena, Italy; Thoracic Surgery Unit, University of Siena, Italy

Key words
Bronchial hamartomas • Mucosal overgrowth • Pulmonary neoplasm

Summary
Multiple hamartomas of the lung are uncommon. The few cases described to date are multiple parenchymal hamartomas or multiple endobronchial and parenchymal hamartomas. Herein, an original case is described which is characterized by the association between an endobronchial hamartoma and multiple, unusual, bronchial lesions of the peripheral lung.

Introduction
Hamartomas are an abnormal mixture of tissue elements, or an abnormal proportion of a single element, that are normally found in an organ. They may be linked to genetic syndromes, such as Carney’s complex, and Cowden’s disease. Pulmonary hamartomas are divided into parenchymal and central endobronchial types. Twenty cases of multiple hamartomas of the lung have been reported in the literature. Almost all of these were multiple parenchymal hamartomas, three were chondromatous, endobronchial and parenchymal, and two were multiple chondromatous endobronchial hamartomas. Herein, we describe an interesting case of bronchial fibrolipomatous hamartoma associated with multiple, unusual, bronchial lesions of the peripheral lung.

Case report
A 51-year-old man was admitted to the Thoracic Surgery Unit of Siena University Hospital in October 2007 with a persistent cough. He smoked 60 cigarettes/day and was working as a plastic printmaker. He suffered from non-insulin dependent diabetes and had high serum triglycerides. A CT scan revealed a 3-cm-pulmonary nodule with lobulated margins near the origin of the right lower bronchus. Bronchoscopy showed an endobronchial, non-ulcerated lesion with smooth margins obstructing the lumen of the apical segmental bronchus of the right lower lobe. It was suspicious for malignancy. A biopsy was performed, but the histological diagnosis was inconclusive. The patient underwent right lower lobectomy. He was subsequently given genetic counselling and underwent clinical evaluation to exclude Cowden’s syndrome, with negative results.

Materials and methods
The pathological specimen consisted of the right lower lobe measuring 12 x 8 x 5 cm, and mediastinal lymph nodes. Serial sections were cut from formalin-fixed tissues, perpendicular to the bronchial airways, and were macroscopically examined. The lesions and the pulmonary parenchyma at different sites were sampled. Histological sections were stained with haematoxylin and eosin, and with Weigert, Gram, Grocott and Masson stains.

Results
Macroscopically, a well-circumscribed, whitish, elastic, endobronchial, polypoid lesion was observed, measur-
ing 0.5 cm, which partially obstructed the segmental apical bronchus at its origin (called the central lesion) (Fig. 1a)
Throughout its branches, as far as the most distal parts of the lobe (not only in its apical segment) the bronchial tree was characterized by patchy, irregular, pseudonodular thickening of the bronchial wall and distortion of the lumen in which mucosal overgrowths and an amorphous whitish material were present (called the peripheral lesions) (Fig. 1b-d). The pulmonary parenchyma did not show any pathological changes.
Microscopically, the central lesion was attached to the bronchial wall with a narrow peduncle, and was mainly composed of fibro-adipose tissue with no evidence of chondroid or smooth muscle elements (Fig. 2).

The peripheral lesions, measuring from 0.2 to 0.5 cm, consisted of irregular and thickened bronchial walls deformed by disorganized mesenchymal components (cartilage, fat, smooth muscle and fibrous tissue), the most prevalent being cartilaginuous tissue. The bronchial lumen was distorted and often contained amorphous material and cellular debris (Fig. 3a-b). The bronchial epithelium was hyperplastic and bundles of elastic fibres were evident in the corion (Fig. 3c-d).

Discussion
We describe a case of bronchial fibrolipomatous hamartoma associated with multiple, peculiar, bronchial lesions of the peripheral lung which differ, in several aspects, from those previously described in the literature. The central endobronchial fibrolipomatous lesion was associated with peripheral lesions which were represented by irregular bronchial overgrowths without luminal polypoid protrusions. The discrepancy between the CT measurement of the central lesion (3 cm) and its measurement on the gross specimen (0.5 cm) is probably due to the fact that the CT included bronchial changes.
The mesenchymal component of the central lesion was mainly fibroadipose, thus suggesting a diagnosis of fibrolipomatous hamartoma, while the lesion found at the periphery was mainly chondroid. The cases described in the literature are characterized by multiple endobronchial and parenchymal hamartomas all of which consist of the same tissues\(^{14}\), with the adjunct of adipose tissue in endobronchial lesions\(^{15}\).

In our case, the peripheral lesions were not parenchymal but bronchial, and were characterized by disarrayed mesenchymal components within the bronchial wall. Although such lesions may be included in the definition of hamartoma (according to the WHO classification\(^1\)), they could not be allocated to either the endobronchial or the parenchymal types described in the literature. In fact, they do not have endobronchial polypoid growth, nor are they round, chondroid, peripheral nodules without any associations with airways.

Finally, these peripheral bronchial lesions seem to be malformations rather than benign neoplasms\(^9\), as hamartomas are currently defined, and can hardly be related to bronchial obstruction since they are not localized beyond. Notwithstanding the absence of frank inflammation, it cannot be excluded that these lesions may be reactive.

In conclusion, the case illustrated here is somewhat unusual and cannot be allocated to any category of multiple hamartomas described to date. Further studies are necessary to understand the incidence and nature of such lesions.

References

Giant genital cavernous haemangioma: case description and surgical management

G. SARTORI*, C. DE GAETANI*, G. BIANCHI
Department of Urology, University of Modena and Reggio Emilia, Italy; *Department of Pathology, University of Modena
and Reggio Emilia, Italy; †Department of Reconstructive Surgery, University of Modena and Reggio Emilia, Italy

Summary

Giant genital haemangiomas are rare occurrences. Once properly diagnosed, they should be managed by surgery with wide and deep margins. We present a clinical case and provide suggestions for diagnosis and treatment of this unusual pathology.

Introduction

Haemangiomas are benign vascular malformations mainly described in children. Although their incidence is around 8-12% in neonates and increases in pre-term newborns, the condition is a rare event in adults. Haemangiomas consist of an enlarged dysplastic vascular tissue with anomalous growth of the endothelial cells. They are divided into capillary, cavernous, arteriovenous, venous and mixed histological variety, with the cavernous and mixed being the most common ones. Beyond the rarity of malformations in the genital area (less than 1%), haemangiomas can present with an intrascrotal or penile location, but only a small number extend to the closest tissues as perineum or abdominal wall. We describe an uncommon case of a diffuse haemangioma involving the scrotum, penis and deep perineum tissues.

Case report

A 51-year-old man came to our Department complaining about the recent onset of mild scrotal pain. He referred a 20 year history of penile and scrotal tender swelling, previously diagnosed as haemangioma based on its clinical appearance. It consisted of soft nodular tissue, easily compressible without provoking significant pain. No other symptoms were recorded, and the remaining the patient history was unremarkable. MRI of the pelvis was performed, which suggested a higher extension of the mass than observed by clinical examination (Fig. 1). In fact, beyond superficial spread, the vascular...
Malformation was widely diffused to the perineum and pelvis, reaching and surrounding the prostate, seminal vesicle and rectum. Those lesions were hypointense on T1-weighted images and hyperintense on T2-weighted images. A subsequent abdominal CT was performed to exclude a systemic origin or involvement of the vascular mass, which was non-contrast enhanced but with small multiple calcifications. Based on these radiological features and the presence of symptoms, we decided to manage the penile, scrotal and perineal nodules with surgical excision. After a previous delimitation of the scrotal haemangiomatous mass, venous nodules were progressively isolated and ligated, and a 15-cm specimen was finally retrieved. A scrotal drainage was left in site for two days, and the overall postoperative period was uneventful. At a 2-months clinical follow up, the patient reported an adequate aesthetic and clinical outcome without the presence of symptoms or complications. Macroscopically, the tumour presented as a soft, spongy, irregularly lobulated mass (Fig. 2); histologically, it was an unencapsulated proliferation of large dilated vessels varying in thickness and lined by flat endothelial cells. Areas of small closely-packed capillary-type vessels filled by erythrocytes were detected within connective tissue and smooth muscle boundless. No mitotic figures and no pleomorphism was detected (Fig. 3). Diagnosis of cavernous haemangioma was made.

Discussion

Genital haemangiomas are an uncommon but well described pathology. Since its first report dating back at 18513, several cases have been documented; in a review, Ruiz Liso et al. found a total of 54 cavernous haemangiomas with scrotal location reported3. Haemangioma represents the most frequent type of vascular mass, as malignant differentiation is quite rare23. Clinically, they manifest as either a faint blue patch or a soft vascular nodule2. Ulceration of the overlying skin and tissues represents a rare event1, whereas some cases may present with thrombosis or ischemic consequences3. Genital haemangiomas are often painless, and only 31.5% of patients presenting with symptoms or discomfort3: Erdag et al. have invoked the filling of vascular spaces and consequent activation of the perivascular nerves as an explanation of pain development4. Correct imaging is mandatory as some haemangiomas may clinically reproduce other paratesticular and scrotal masses as well as inguinal hernias2. Scrotal ultrasonography represents an important diagnostic step as it reveals the content of the lesion, and Color Doppler may reveal blood flow that is often present in vascular masses. Our clinical case demonstrates that further imaging may be required to assess the extent of the haemangioma, which can potentially involve deep tissues or organs2. In this respect, MRI is a non-invasive tool to investigate scrotum and penile shaft, and haemangiomas typically present as soft lobulated masses hypointense on T1- and hyperintense on T2-weighted images. CT scan of these vascular masses reveals intense enhancement on postcontrast images, together with calcifications; moreover, CT is advised to detect any associated abnormalities or other pathological conditions2. Before planning an interventional approach, imaging represents a crucial point, as some haemangiomas can extend widely, as was evident in the presented case. M. Froehner et al. described the case of giant cavernous penile hemangioma with intrapelvic extension5; the possible association with mega penis and agenesis of the corpus spongiosum was reported by Y. Nouira et al.6. Recently, a giant scrotal and penile haemangioma have been described with extension to the rectum, provoking episodes of bloody stool2. Eradication should be recommended and treatment op-
tions include sclerosing agents, laser therapy and surgery. Even if laser therapy is associated with a high success rate (92.8%), we believe that surgery is the treatment of choice to deal with difficult and complex cases like the present since it can provide satisfactory clinical outcomes without significant morbidity.

In conclusion, despite the rarity of genital haemangiomas, their occurrence must be promptly recognized, and complete diagnosis requires accurate imaging to assess extension. Once properly detected, surgical management represents the treatment of choice to deal with giant genital haemangiomas.

References

Pulmonary lymphangioleiomyomatosis in tuberous sclerosis. A case report

N. DE ROSA
U.O.C. di Anatomia Patologica, A.O.R.N. Monaldi, Naples, Italy

Key words
Pulmonary lymphangioleiomyomatosis • Lymphangiogenesis

Summary
Pulmonary lymphangioleiomyomatosis (PLAM) is a rare disease that exclusively affects young women of reproductive age. It is characterized by widespread pulmonary proliferation of abnormal, “immature smooth muscle cells (lam cells) leading to cystic destruction of the lung parenchyma. Lam occurs frequently in the thoracic duct and in axial lymph nodes, mediastinal or retroperitoneal. It can occur either in association with tuberous sclerosis complex (TSC-LAM) or without TSC (sporadic LAM). A case of TSC-LAM is reported, and the histogenesis and the role of lymphangiogenesis in the progression of disease is discussed.

Introduction
Lymphangioleiomyomatosis (LAM) is a rare, multisystem disease characterized by widespread proliferation of abnormal smooth muscle-like cells (LAM cells) in the lung and/or in axial lymph nodes and/or in other organs. LAM is frequently complicated with renal angiomyolipomas. Although two cases involving male patients were recently reported, one of which in association with the tuberous sclerosis complex (TSC), it occurs almost exclusively in women, generally during reproductive years and seems to be hormonal-dependent as it worsens during pregnancy and menses or following oestrogen therapy. Oestrogen or progesterone receptors have been identified by immunohistochemistry in pulmonary lesions. The most common symptoms are shortness of breath, expectoration of bright red blood and cough. The clinical signs are repeated pneumothoraces, and chyloous effusions. The functional features are variable and show either obstructive or restrictive aspects with increased total lung capacity and decreased diffusing capacity. In approximately 15% of women, PLAM is tuberous sclerosis complex (TSC)-related. In this report, a new case of PLAM is presented and its occurrence in TSC is highlighted.

Case report
A 30 year-old woman, in a good state of health, presented a spontaneous left pneumothorax (PNX) treated with endopleuric drainage. After 30 days, the PNX recurred. After the resolution of the PNX, the patient was submitted to a thoracic CT scan that revealed a diffuse pulmonary cystic pathology, with thin walled cysts measuring a few millimetres to about 2 centimetres, evenly distributed throughout all lung zones. The abdominal CT scan revealed the presence of multiple bilateral renal angiomyolipomas and the presence of an adipose tissue density lesion in the VIII hepatic segment, compatible with a diagnosis of angiomyolipoma. Laboratory data and functional respiratory aspects were normal. Video-assisted thoracoscopic atypical lingular biopsies were performed. Pulmonary biopsies were fixed in 10% neutral buffered formalin and embedded in paraffin. Haematoxylin and eosin stained sections were examined by light microscopy. Additional sections were obtained for the immunohistochemical study, utilizing antibodies against cytokeratin, vimentin, S100 protein, human melanin black-45 (HMB45), specific muscle actin (HHF35), smooth muscle actin (1A4), CD1a, estrogenic receptors (ER), progesterone receptors (PR). All the antibodies were prediluted and supplied by Ventana. The immunostaining was performed using a Ventana Bench-Mark XT Autostainer following the manufacturer’s instructions. Histologically, lung tissue was characterized by areas with cystic changes and heavy thickening, sometimes in a nodular fashion, of the alveolar walls, due to the infiltration of spindle-shaped cells with pale eosinophilic cytoplasm (Fig. 1). Within the micronodules, the spindle cells were divided into fascicles or bundles by slit-like lymphatic vessels; the spindle cells were also observed...
in the thin walls of cystic spaces. An adjunctive finding was the hemosiderosis. Immunohistochemically, lesional cells were strongly positive for smooth muscle actin (Fig. 2), whereas some cells reacted with HMB-45 (Fig. 3). Furthermore, receptors for oestrogen and progesterone were weakly positive (Fig. 4).

The histological diagnosis was pulmonary lymphangioleiomyomatosis and the histological score according to Matsui was rated as 2. On the basis of the concomitant presence of PLAM, multiple bilateral renal angiomyolipomas and radiological hepatic lesion compatible with angiomyolipoma, each of which is a major criteria for diagnosis of TSC (Tab. I), the patient was considered to suffer from tuberous sclerosis. She was submitted to bilateral ovariectomy. At present, after three years, she has a slight respiratory insufficiency.

**Discussion**

Pulmonary lymphangioleiomyomatosis (PLAM) is an entity whose nosological classification is difficult. It may be included into the group of the diffuse parenchymal lung diseases which predominantly involve the “alveolar interstitium”, since both clinically and radiographically it poses problems in the differential diagnosis with other interstitial diseases. The natural course of the disease leads to a severe respiratory insufficiency over a period of several years. The most common symptoms are shortness of breath, expectoration of bright red blood and cough. The clinical signs are repeated pneumothoraces and chylous effusions, all of which are explained by physiopathological features of the disease: the obstruction of bronchioles by proliferating “abnormal” smooth muscle cells leads to airflow entrapment by a valvular mechanism. Cyst formation is due to the degeneration of interstitial connective tissue by the protease action of LAM cells.
are subtle. The clinical signs of TSC are numerous. The most frequently considered are included into the triad of Vogt: convulsions, mental retardation and facial angiofibromas, but other signs are not negligible. Gomez has developed a scheme of hierarchically organized symptoms and signs subdivided in major and minor criteria (Tab. I) to better classify these patients. Following this scheme, the presence of one of the major criteria is virtually diagnostic of TSC, whilst the presence of a minor criterion is suspicious; the presence of two minor criteria is presumptive for diagnosis of TSC.

In the present case, the patient showed multiple and bilateral renal angiomyolipomas, corresponding to a major criterion of Gomez, and thus diagnostic of TSC. The presence of PLAM is a minor criterion of Gomez, and if isolated is only presumptive. The coexistence of a major and minor criterion reinforces the diagnosis. PLAM is included in the family of pulmonary and extrapulmonary lesions composed of perivascular epithelioid cells (PECs), called PEComas. They are related to the genetic alterations of the tuberous sclerosis complex. PEC presents an unusual and characteristic phenotype as it co-expresses both contractile proteins and melanocytic markers, such as actin and HMB45, and can modulate its appearance giving rise to various morphologic patterns from epithelioid to spindled 10. No normal counterpart of this cell has been identified.

Histologically, the full-blown cases of PLAM rarely present diagnostic difficulties. In subtle cases or in cases with limited tissue, HMB45 staining can be helpful for highlighting modified smooth muscle cells. The main differential diagnosis is with advanced interstitial fibrosis, showing reactive hyperplasia of bronchiolar and vascular smooth muscle. A further condition that enters into differential diagnosis is the so-called benign metastasising leiomyoma. In the above cited diseases, the proliferating smooth muscle cells are devoid of the bi-phenotypic differentiation and do not express HMB45.

Recently, a new useful immunohistochemical marker has been described, namely the papain-like cysteine protease cathepsin-k, which is useful for diagnosis in difficult cases. 17. The clinical course of the disease is usually progressive, and approximately 30-70% of patients die within 10 years of diagnosis.

Several therapeutic strategies have been attempted including hormonal manipulation, oophorectomy and Tamoxifen, but the success rate has not given promising results. Lung transplantation has been performed in some patients, but recurrence of LAM in transplanted lung has been reported. 18. This aspect, as far as the progression of disease because of the dissemination of LAM cells into systemic circulation with the subsequent formation of new lesions, can be explained by lymphangioigenetic activity of the same LAM cells and by the action of matrix metalloproteases 19,20 and papain-like cysteine proteases such as cathepsin-k 17.

Recent molecular genetic studies in sporadic angiomyolipomas and in other forms of PEComas indicate that TSC1/TSC2 functions as a negative regulator of the

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Tab. I. Gomez’s criteria for diagnosis of tuberous sclerosis.

<table>
<thead>
<tr>
<th>Major criteria *</th>
<th>Minor criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple angiomyolipomas of the kidney</td>
<td>Multiple tumours or cysts of the kidneys</td>
</tr>
<tr>
<td>“Tubers” of the cerebral cortex</td>
<td>Pulmonary lymphangioleiomyomatosis</td>
</tr>
<tr>
<td>Subependymal glial nodules</td>
<td>Cardiac rhabdomyomas</td>
</tr>
<tr>
<td>Retinal hamartomas</td>
<td>Tuberous sclerosis in relatives</td>
</tr>
<tr>
<td>Angiofibromas of the face</td>
<td>Infantile spasms, convulsions, calcification of the CNS</td>
</tr>
<tr>
<td>Ungual fibromas</td>
<td>Cutaneous hypomelanotic spots</td>
</tr>
</tbody>
</table>

* The presence of one major criterion is diagnostic † The presence of a minor criterion is suspicious, and of two minor criteria is presumptive.
Rheb/mTor/p70S6K signalling pathway. The mutation of these genes dysregulates S6K1 activation, which leads to the abnormal cell proliferation associated with LAM disease. Further investigation of these metabolic/regulatory phenomena may lead to the development of new therapeutic strategies such as specific m-TOR inhibitors like rapamycin for improvement and/or prevention of disease progression in LAM.

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5° Simposio Nazionale di Citopatologia
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INDEX / INDICE PER ARGOMENTI

Lectures / Relazioni

International session / Sessione internazionale  page 193

Session I: Cervicovaginal cytopathology and screening / I Sessione: Citopatologia cervico-vaginale e screening  » 193
Session II: Molecular cytology / II Sessione: Citologia molecolare  » 195
Session III: Teaching Cytology in Italy / III Sessione: L’insegnamento della Citologia in Italia  » 196
Session IV: Thyroid / IV Sessione: Tiroide  » 196
Microembedding (Microhistology) in Cytopathology / La Microinclusione (Microistologia) in Citopatologia  » 197
Special session: Initial screening in cervicovaginal cytology / Sessione per primi lettori di citologia cervico-vaginale  » 199
Session V: Cytopathology in Breast Pathology / V Sessione: Citopatologia in Patologia mammaria  » 200
Round table: Can urinary cytology be improved with new technologies? / Tavola Rotonda: Si può migliorare la citologia urinaria utilizzando le nuove tecnologie?  » 202
Practical cytopathology: how to improve diagnosis and experience / Citopatologia pratica: come migliorare la diagnosi e l’esperienza  » 203

Short course: Endometrial cytology / Corso breve: Citologia endometriale  » 205

Free presentations / Comunicazioni libere  » 206

Author index / Indice per autori  » 225
The future of cytology in cancer prevention

C. Bergeron
Laboratoire Pasteur-Cerba, 95066 Cergy Pontoise Cedex 9

Cervical cancer is one of the rare cancers where there is easy access to the organ and the screening test is simple and relatively cheap. In addition, the slow progress of the disease means that there is a long period of time to detect precancerous lesions before they become invasive. The Pap screening has been very successful. However, this test is also limited in terms of its sensitivity and its low reproducibility. Even in developed countries the coverage is never 100%. In developing countries, the coverage is very low because of a lack of resources and a lack of pathologists and cytologists.

The HPV 16/18 vaccine has been developed thanks to virus-like particles (VLPs) which are non-infectious but which provoke the production of neutralising antibodies. The duration of the protection seems to be very good so there may be no need to do a repeat injection. However, the current vaccines only provide protection against 70% of cervical cancers. Also, they do not protect women who are already infected by HPV 16 or 18. This implies vaccinating much earlier than the average age of the first sexual experience. If women are vaccinated between 10 and 15 years old, the time necessary to begin to see the impact on this vaccinated population on the incidence of cervical cancer will be at least 20 years. The time necessary to see the total impact of vaccination, with the entire generation of women having been vaccinated, will be closer to 30-50 years. It is therefore evident that there can be no question of ending screening when vaccination is introduced because there will be a time shift in the impact of a vaccination programme of at least 20 years. This impact will also greatly depend on vaccination coverage. The impact on cervical screening programme by cytology will be much earlier and depends of the catch up population concerned by the vaccination programme (16-25 years). The vaccination will decrease the percentage of abnormal smears with lowering the probability of high-grade lesions but not much low grade lesions or minor atypia. Then cytology will be more prone to loss of accuracy. Then, for the young vaccinated women, primary HPV testing with triage by cytology and prolonged screening interval would be probably the best scenario. Pap screening or primary HPV screening for older non vaccinated women remains a debate. HPV screening on self sampled material in women who have not routine Pap screening could become more important. Cervical cancer prevention can be obtained in the future with the synergy of prophylactic vaccination for the young and adapted cervical cancer screening for the older women. Organized programme will permit to control the coverage, adapt the test and the interval to the age and follow appropriately the positive cases.

Session I
Cervicovaginal cytopathology and screening

Screening con HPV: lo studio NTCC

G. Ronco
CPO Piemonte, Torino

HPV testing is cross-sectionally more sensitive than cytology in detecting high-grade cervical intraepithelial neoplasia (hgCIN). Two randomised controlled trials (RCT) observed decreased detection of hgCIN after screening by HPV testing than by cytology.

The New Technologies for Cervical Cancer screening (NTCC) study was designed in order to compare the occurrence of hgCIN after screening with HPV DNA and after cytology as primary tests.

In a randomised controlled trial, women were randomly assigned to a conventional arm (conventional cytology) or to an experimental arm (HPV testing + liquid-based cytology in phase one and alone in phase 2). Women testing negative in each arm were re-invited after 3 years for a new screening round with conventional cytology. We studied the detection of histologically confirmed CIN2+ in the two arms after recruitment.

Some 22,547 and 24,353 women were enrolled in the conventional during phase one and phase two respectively. Women enrolled in the experimental arm were 22,708 in phase one and 24,361 in phase two. Concerning women recruited during phase one, at the new screening round, there was a reduction of the detection rate of histologically confirmed CIN2+ in the experimental, compared to the conventional, arm (relative DR 0.63; 95% CI 0.32-1.24) and especially of CIN3+ (relative DR 0.24; 95% CI 0.07-0.86). This reduction was stronger among women aged 35-60 than in younger women. We are analysing data from the entire groups (including those testing positive at recruitment) during the two phases.
Screening for cervical cancer worldwide: how does it change in the vaccine era?

G.R. Montanari

CPO Piemonte

Worldwide, cervical cancer causes 493,000-510,000 new cases and 274,000 deaths. Its incidence is second only to that of breast cancer. Similarly, among European women aged 15-44, it is the second cause of death from cancer, surpassed only by breast cancer. Every year, in Europe, women diagnosed and dying of cervical cancer are approximately 33,500 and 15,000. In non-screened populations of industrialized countries, the highest annual incidence rate is in the order of 20/100,000. The corresponding rate in Africa, India and Latin America is between 2 and 5 times higher. Cases diagnosed yearly in Africa, Latin America and Asia are respectively 68,000, 77,000 and 245,000.

Incidence of cervical cancer is increasing. In 2006, on the basis of 493,000 cases occurring in 2002, Parkin et al. estimated increments of 19% for 2010 and 42% for 2020, corresponding respectively to 584,000 and 702,500 cases. Fortunately, this trend can be hindered by the expansion of organized screening programmes, whose design may differ between developed and developing countries. Primary prevention through HPV vaccines can also play a role.

It is estimated that HPV types 6, 11, 16 and 18 cause 75% of cervical cancers in Europe. Nevertheless, evaluating the impact of the vaccine on the incidence of cervical carcinoma will require many years. Presumably, offering vaccination programmes to the population will not replace the need for an adequate offer of screening tests. Thus, efficacious and extended secondary prevention programmes should persist, as well as projects for the integration of the two preventive strategies (HPV vaccine and screening). Such integration is needed not only for the benefit of women, but also in order to regulate the use of human and financial resources, which are not unlimited. The problem is how efficiently reach all women without inequality.

The pros and cons of vaccine. The vaccine against some HPV types is on of the most sensational recent achievements in cancer prevention. The only previously known anticancer vaccine was addressed against the hepatitis B virus and, therefore, liver cancer. Currently, HPV cannot be cultivated and cannot be administered in a an attenuated form.

The following are some reasons for concern:

– Given the current cost, the vaccine is mainly offered to wellbeing populations having access to screening programmes (either organized or spontaneous). It can hardly have an impact on the problem of the high incidence and mortality in developing countries, thus creating inequality.

– In the Italian context, it is to be wondered whether a vaccination plan would reach the women who most need it, i.e. those who do not participate to screening programmes, either organized or non organized.

– Follow up of trials implemented in Asia, Europe, Latin and North America has been too short: In most instances, efficacy of the vaccine regard has been verified at most over a period of 5 years.

Other topics requiring clarification. The vaccine only protects vs. HPV 16-18 (and 6-11). The prevailing HPV types are not the same everywhere. If the vaccine included another 8 high risk HPV types, protection would reach 71-83% women. Harper has shown a cross-protection for the current vaccine also for HPV types 31 e 45. Data produced in September 2007, by the studies Future I & II suggest a cross-protection with regard to cervical histological changes which may prevent 38% di CIN II-III caused by 10 HPV types additional to types 16-18, i.e. types. 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, which are responsible for about 16% cervical cancer cases in Europe.

What is not equally clear is the extent to which the vaccine prevents a new infection in time and/or the persistence of the infection by the same HPV type. The latter situation creates a cancer risk for women in their 40s, whereas in younger women HPV infection, albeit very common, spontaneously regresses in 60-80% cases.

Should the first level screening test (HPV test) be modified in vaccinated women? And in those who are not vaccinated?

Can liquid phase cytology be suggested as second level test? This test is already carried out with the viral test “test reflex”: it can be easily used with the advantage that there is no need for requesting the women to return to the laboratory. Would it be reasonable to preserve colposcopy as third level test. The creation of regional vaccination registers coordinated with the call-recall system is most important.

Side effects of the vaccine have probably been overstressed by non scientific publications. However, they require to be evaluated.

In 2008, the American Cancer Society (ACS) issued some recommendations with regard to the use of HPV vaccine (8):

– It is necessary to continue screening for cervical carcinoma according to the current schedule.

– The vaccine may cause a 90% reduction of HPV 16-18 caused abnormal pap tests and a 50% reduction of all abnormal pap tests.

– Nevertheless, it should be emphasized that, in cervico-vaginal cytology, the decrease of the prevalence of CIN II+ causes an increase of VPN, but a decrease of VPP. Thus, monitoring the performance of Pap-test is most important as well as drawing its consequences having in mind possible changes of the guidelines for screening.

References

Session II
Molecular cytology

Chairmen
D. Beccati (Ferrara), A. Cavazzana (Massa)

Molecular biology in the diagnosis of HPV

G. Collina, P. Pierotti
U.O. di Anatomia Istologia Patologica e Citodiagnostica, Ospedale Maggiore, AUSL Bologna

Since the mid-1990s there has been an increasing interest in the use of HPV DNA as a cervical cancer screening tool based on the idea that standardized molecular testing of exfoliating cervical cells for HPV which is responsible of cervical cancer could have a good diagnostic performance and it could be more reproducible than conventional cytology. Many studies suggested the utility of conventional cytology plus HPV DNA testing compared to the cytological test alone as a tool for cervical cancer screening. Most studies have used the Hybrid Capture (HC2) system. It detects the presence of 13 types of HPV that have been associated with cervical cancer. It has been reported that women with a negative Pap test and a negative HC2 test have a decreased risk of high grade lesions when compared with women which have a negative Pap test only. On the other hand, a positive HC2 test is not an absolute indicator that high grade lesion exists or will develop. Nevertheless the prognostic value of a positive HC2 test results in absence of cytologic abnormalities is not fully understood. HPV DNA testing has a greater sensitivity than cytology for detecting clinically relevant lesions. Restricting screening to women aged 30 and older reduces the number of women to be referred to colposcopy due to transient HPV infection. The high negative predictive value resulting from the combined use of conventional cytology and HPV DNA testing could safely permit increasing screening intervals thus lowering costs. Only testing for high-risk HPV would be of value. Testing for low risk HPV types is not useful and may have a negative psychological impact on patients. For this reason one of the major problem in the screening programs for carcinoma of the cervix is the treatment of low grade SIL.

It is well known that, according to the Bethesda System, the presence of koilocytes in a PAP smears allows the diagnosis of low grade SIL. Sometimes cervical biopsies from patients with such a diagnosis fail to show definite dysplastic changes being the presence of bona fide koilocytes the only abnormality. Different ancillary technologies have been used to overcome this problem: immunostiochemistry using antibodies anti-HPV and p16, in situ hybridization with specific HPV-probes, hybrid capture 2 test on liquid based cytology, and MY09/MY11 or GP5+/GP6+ PCR.

We evaluated a series of cases of low grade CIN and high grade CIN from formalin fixed paraffin embedded tissues. Most of the cases with the diagnosis of low grade CIN had a previous ASCUS or low grade SIL on Pap test HPV DNA evaluation was done using MY09-MY11 and Gp5+/Gp6+ primers (L1 region). Positive cases were genotyped by direct sequencing. Negative cases were tested by E6/E7 PCR using primers described by Sasagawa et al.

We found that biopsies from cases of high grade dysplasia harboured high risk HPV viruses while cases showing only koilocytosis had either the presence of low risk HPV viruses or were negative for HPV viruses.

These results suggest that genotyping HPV virus may play a role in distinguishing a subset of patients which deserve a strict follow up and not aggressive treatment.

HPV testing may be used in triage of patients with ASCUS. The reason that HPV testing is such a powerful adjunct to cervical cytology is that it clarifies risk: women (21 years or older) with HPV-ASC-US are at exceeding low risk of precancerous lesions and can return to routine screening without further clinical intervention while women with HPV+ASC-US have a risk comparable to that of low SIL cytology.

With HPV testing, it is possible to weight the risk of having a significant cervical disease against the cost of unnecessary intervention or arm.

Many problems linked to HPV infection would be solved by HPV vaccination program which should target populations with will glean the greatest benefit for the cost: girls in early adolescence who are most naive to HPV infections.

References
Session III
Teaching Cytology in Italy

Chairmen
S. Prandi (Reggio Emilia), A. Vetrani (Napoli)

Masters in Cytopathology
M.R. Giovagnoli
Sapienza University, Rome

From the '70 to the '90 many different schools offered specific courses (lasting from six months to 2 years) to create experts in cytopathology. These courses were open to students with different background (either MD, student with bachelor degree in biology or with technical degree) and it has been calculated that more than 2000 people performed such courses. However their legal value was never recognized at normative level, even if the necessity of the “cytologist” as a specific professional figure was advocated by the National Oncology Committee (GU 127, 1/06/1996) and by the Stato-Regioni Conference (GU 102, 2/5/2001).

As the University rules changed, according to the European Sorbona protocol, the previous specific courses in cytology had to stop. The possibility of academic courses, according to the new European rules, consisted mainly in University Master courses as a response to the need for permanent formation of the modern society. The huge amount of new Master Courses offered by the Italian University, even if recognised as academic degrees, at present, have no impact on the evaluation of candidates who apply for specific employments.

In an effort to fill in the gap in institutional training and updating on cytoscreening subject, some Universities set up a first level Master Course in “Diagnostic Cytopathology in the screening of the population” as from the academic year 2003/2004 (Sapienza University, Rome) and 2004/2005 (Turin University). These courses were attended by about one hundred students.

These courses last for one year and have been followed both by beginners and by experts working in the field of cytopathology. Specific interviews on the Master Course have been presented to the students and a positive reception of the course has been demonstrated. Some students could also get a job as a consequence of these masters.

The important issue of an official recognition of this Master for job application in cytology has been emphasized by many of the participants and it is still matter of debate. Only recently the institution of “Professionalizing Masters” has been proposed within the National Conference for Medical Profession held in Ancona.

Previous efforts to have an official legal recognition of cytology and cytopathological screening as a specific and well recognized task in the field on medical professions has failed. Recently within the Cytopathology Group of the SIAPEC, Professor Navone proposed a National Aptitude Test similar to the European EFCS-QUATE Aptitude Test in Cervical Cytology, which is recognized by Scientific Societies. The first one will be held in Turin just before the next Cytological Symposium in November, and a second one will follow in Rome the same month.

This test will be offered to all students participating to Masters in Cytopathology with the aim of sharing common rules with other countries of the European Union.

Of course, training in cytology for pathologist is not limited to cervical screening but should be part of the Surgical Pathology School. As detailed training is not offered by each Postgraduate Medical School, perhaps specific Masters in different fields of Cytopathology could be offered by creating a teaching-net within different Universities and with the involvement of Hospital Departments (with specific cytopathological expertise) connected to Universities.

Session IV
Thyroid

Chairmen
P. Boccato (Padova), G. Bussolati (Torino)

Immunohistochemical malignancy-related markers in indeterminate FNA thyroid lesions
M. Volante, A. Fornari, M. Papotti
Department of Clinical and Biological Sciences, University of Turin, Italy

Thyroid nodules represent a common clinical problem. The prevalence of palpable thyroid proliferations in adults increases with age, with an average of 4-7% for the United States population but higher in iodine-deficient areas where subclinical nodules are frequently incidentally discovered following thyroid ultrasound-scan. More than 90% of these thyroid proliferations are benign and for this reason a reliable and systematic approach to their evaluation represents an important task to be pursued for avoiding a surgical over-treatment. Ultrasound-guided fine-needle aspiration biopsy (FNAB) is the gold standard for thyroid nodule evaluation, but it is widely known that this method has some intrinsic limitations to correctly distinguish between benign (i.e. nodular hyper-
plasia and adenoma) and malignant follicular lesions (i.e. follicular thyroid carcinoma and follicular variant of papillary carcinoma), with a diagnostic failure of up to 30% according to different centres. As a consequence, those follicular thyroid nodules that remain indeterminate at thyroid FNAB cytology (classified TIR3 according to recently proposed SIAPEC guidelines) are referred to surgery more for diagnosis than for therapeutic necessity, and less that 10% of such cases will prove to be malignant at final histology. To reduce the number of follicular proliferations referred to surgery, and therefore to reduce costs for public health, several immunocytochemical markers have been proposed to distinguish malignant from benign follicular proliferations. The use of immunocytochemical markers on FNAB material may be generally employed on smears, although cell block preparations seem to be more reliable in this specific setting. The markers proposed are mainly related to tumor-associated abnormal expression of cellular antigens, such as cell surface mesothelial antigen HBME-1 (HBME1), cytokeratin-19 (CK19), thyreoperoxidase (TPO), keratan sulphate (KS), or to the specific expression of cell-cycle or apoptosis related molecules, such as galectin-3 (Gal-3), or oncogenes, such as RET. As a general comment, it is generally advisable to rely not on a single marker but rather on a combination, to achieve the best specificity and sensitivity. The role of one of the most employed markers, GAL-3, has been recently validated in a large prospective multicentric study from an Italian population and confirmed an overall sensitivity and specificity of this immunocytochemical test of 85% and 93%, respectively, with estimated positive and negative predictive values of 83% and 94% respectively. More than 91% of indeterminate (TIR3) follicular thyroid nodules enrolled in this study were considered correctly classified preoperatively. However, despite the strengthened experience of different groups, the use on immunocytochemical markers of malignancy in indeterminate FNAB lesions is not accepted worldwide, at least to reach a consensus on their standardized used.

References

Microembedding (Microhistology) in Cytopathology

Chairmen
A. Leotta (Lamezia Terme), R. Navone (Torino)

Microembedding in thyroid cytology

S. Asiolli, D. Pacchioni, F. Maletta, G. Accinelli, G. Bussolati
Department of Biomedical Sciences and Oncology, University of Turin, Italy

Fine needle aspiration (FNA) of the thyroid, which is a rapid and cost-effective procedure, has gained wide acceptance as a valuable method for distinguishing neoplastic from non-neoplastic nodules and identifying those patients requiring surgery. At the present time, the thyroid gland is the most frequently aspirated organ for triage/diagnostic purposes.

Clinically-relevant thyroid nodules occur in 5-10% of the general population in Italy and approximately 5% of the patients have malignant lesions. The goal of thyroid FNA is to identify the nodules that require surgery and decrease the number of thyroidectomy for patients with benign disease. Overall, the technique has a high sensitivity and specificity for the detection of thyroid neoplasm.

Cell blocks (CBs) are often prepared on FNAs from several organ sites as an adjunct to smears in the diagnosis of potential lesions. However, the literature contains few reports on their utility with regard to specific organ sites. The main advantage of the CB is the potential to produce several sections for special stains and other ancillary, in particular immunohistochemical, studies. At our institution, CBs are made routinely on thyroid FNAs since twenty years and we have been performing about eight-hundred thyroid FNAs each year. As a result, we have been collecting a high number of CB of thyroid lesions, of great value for investigative and retrospective studies.

The aspirates were procured by experienced cytopathologists, clinicians, or radiologists. The majority of the aspirates were performed by the radiologist or clinicians under ultrasound guidance and each aspirate had an immediate in situ assessment for adequacy by the cytopathologist. FNAs were performed using a 23- or 25-gauge needle attached to a 10-ml disposable syringe mounted to a metallic Cameco syringe pistol (Morton Medical Ltd., London, United Kingdom). One to 4 aspirates were performed per case. After each sampling, air-dried Hematoxylin & Eosin-stained slides were made for immediate assessment and alcohol-fixed slides for subsequent Papanicolaou (Pap) staining. The residual material and the needles were rinsed immediately in a 50% Ethanol solution (s.c. Lysis Buffer) containing Ammonia Chloride, Potassium Bicarbonate and EDTA, which proved effective for fixing cells and lysing erythrocytes. Material collected by centrifugation was embedded in paraffin.
Sections prepared from the CBs were evaluated for the presence of cellular fragments. CB has greatly improved the pre-surgical diagnosis of thyroid nodules, since small tissue specimens representative of the lesion were detected in the great majority of cases. These “micro-biopsies” provided morphologically-relevant data. Besides, sections were employed for immunostaining and molecular tests. Expression of galectin-3 is routinely tested on thyroid follicular lesions (cytological cell-blocks) obtained preoperatively by ultrasound-guided fine-needle aspiration of thyroid nodules 10.

Moreover, CB procedure provides the basis for new molecular and immunohistochemical studies to determine the definition of thyroid neoplasm of indeterminate malignant behavior (Thy3). Recently, we focused on the immunohistochecistry of Emerin, a protein of the nuclear membrane (NM) whose decoration best demarcates the nuclear shape of the thyrocytes 11 and we performed this stain on a series of 82 cytological CB thyroid specimens. Emerin revealed a uniform arrangement of the NM in non-neoplastic lesions (thyroiditis, microfollicular goiter, follicular adenoma) and normal thyroid as well as in follicular carcinoma. In contrast, irregular folding of the membrane and presence of curling and invaginations, eventually leading to the formation of nuclear pseudoinclusions, was observed in PTC and VFPTC cells.

In conclusions, the integration of CB method with conventional cytomorphological and clinical diagnostic procedures represents a sensitive and reliable diagnostic approach for preoperative identification of thyroid carcinomas. This procedure improves the diagnostic accuracy of conventional cytology. Moreover, the CB is useful for immunohistochemical and molecular study of thyroid neoplasm.

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Cell Block Sections of FNAB. Immunocytochemistry and Molecular Analyses on Deep-Seated Tumor Samples

A. Fabbri, C. Riva, C. Alphandery, S.A. Colombo, K. Ponzoni, M. Ruggeri, A. Carbone

Department of Pathology and Laboratory Medicine, IRCCS Istituto Nazionale Tumori, Milano, Italy

With advancements in interventional radiology, fine-needle aspiration biopsy (FNAB) has improved our ability to detect small lesions and to obtain suitable tumor tissue for diagnosis from lesions deep-seated in the body. Core needle biopsy (CNB) and FNAB are complementary techniques and are performed routinely in our Institution. FNAB in comparison to CNB is able to provide tumor cells with much lower contamination of stroma than CNB for molecular analysis of tissues. In many cases cytomorphic features alone might be sufficient for making a diagnosis. The use of ancillary tests is often necessary not only for rendering a specific diagnosis but also for determining prognostic and predictive factors. Immunocytochemistry performed on direct smears, monolayered preparation, and cell block sections of FNAB is the most commonly utilized ancillary technique for determining the organ of origin of a metastatic tumor, and for classification and typing of tumors. Most molecular techniques, including in-situ hybridization (ISH), polymerase chain reaction (PCR), and transcriptional profiling, can be performed with cell block sections of FNAB. ISH with chromogenic or fluorescent signals has distinct advantages over other molecular techniques because it allows comparison of cellular morphology with chromosomal alterations in cells. Reverse transcription-polymerase chain reaction (RT-PCR) analysis allows for amplification of very limited quantities of transcripts. This technique is suitable for molecular analysis of limited amounts of material, such as that procured by FNAB. In addition to immunocytochemistry, molecular tests can aid in determining a more specific diagnosis but also for determining prognosis and response to therapy.
Roles and duties of cytoscreener

R. Bio, T. Rubino, L. Campioli, B. Aguzzoni, S. Prandi
Centro di Citologia Cervico-Vaginale di Screening-Arcispedale Santa Maria Nuova di Reggio Emilia, Italy

At the beginning of ’90s the Superior Health Institute, inside the International project of Screening Program, has suggested the utilization of professional figures, used for the first reading of cervico-vaginal cytology, identified as well prepared biomedical laboratory technicians and denominates “Cytoscreeners”. Both European and Italian guidelines have underlined the importance, for the good outcome of an organized screening program, of an adequate preparation of the operators.

An important role as it regards the cytoscreeners formation is due to the introduction in 1990, thanks to the work done from a job team presided by Doctor E. Mc Googan, of the European Competency Test in cervico-vaginal cytology, for the different professional figures dedicated to Pap smear reading (EFCS/QUATE – European Federation of Cytology Societies/Committee of Quality Assurance, Training and Education). This test appraised the preparation of operators assigned to Pap test reading and consigned a “Certificate of competency in gynaecological cytopathology” to the candidates that had overcome the test. This test has demonstrated, during the years, to be an important instrument for the quality control which measures the experience and skill levels in smears reading. The specific professional formation started with sporadic courses given in Italy in the ’70s, in few Institutes (Istituto Tumori in Milan), has been afterwards consolidated with dedicated schools and university masters which unfortunately are not yet legally recognized in Italy.

The European Guidelines on Cytology laboratory and Quality Assurance, recognize two different figures of cytoscreener:

1) the citoscreener having less than 5 years experience with the role to individuate the precursory signs of preneoplastic lesions, to manage the laboratory both as it regards preparation and staining of slides, having also some possible administrative duties;

2) the citoscreener having more than 5 years experience (cytoscreener senior) having responsibility of quality control, administrative, diagnosis and laboratory roles.

The Italian SSN has not yet clearly defined competencies and responsibilities of the citoscreeners and consequently their economical treatment, due to the institutional absence of this professional figure. Nowadays we are in a transition phase in which professional figures of biologists and cytoscreeners are sharing the cytopathological diagnosis. The life in common is necessary because both professional figures result damaged for a lack of legislative clearness. It is to be hoped that, having Scientific Societies support, clarity on professional path of both figures should be done and professional register should be established.

Conclusions. In spite of the numerous steps moved forward in these last years an adequate legal identification of the professional role of cytoscreeners is still missing. For the future is desirable:

1) The establishment of a professional register for laboratory technicians.
2) Recognized compulsory professional course (mandatory professional masters) to be admitted to SSN contest.
3) Dedicated contests with specific tests.
4) Specific duties, separate from the others technicians inside Laboratories of Pathology.
5) Adequate economic award, commensurate to the responsibility level.

References


A seminar on intercontinental cytopathologic casististic managed by telepathology

L. Viberti
Patologi oltre frontiera ONG

The Association “Patologi Oltre Frontiera, ONG” (POF [Pathologists Beyond Borders, NGO]) are organized into a national group that includes the professional roles of pathologists, biologists and laboratory technicians. The main aim of POF is to aid the development of an organized health care in poor countries beyond the management of major health emergencies which also deals with the management of oncologic diseases.

Hence POF wants to achieve this objective through the creation of new laboratories of surgical pathology in developing countries and the training of local health staff. In most of the projects managed by the Association, the possibility of organizing a remote diagnostic support through Internet (the so called “telepathology”) has allowed to compensate the present lack in these countries of specialist in surgical pathology, both pathologists and technicians.

In developing countries, carcinoma of the uterine cervix is the leading cause of cancer death of women and 80% of all new cases worldwide are found in these countries. The Pap test has proven its diagnostic effectiveness in industrialized countries where the incidence of invasive cervical carcinoma has showed a sharp decrease. The same way to detect this carcinoma in an early stage has been proposed by POF in countries where they currently have projects, Collaboration with gynecologists of the Italian Society of Colposcopy has allowed to introduce the colposcopy in a project in Zambia, therefore the ability to perform targeted biopsies and a subsequently treatment of lesions by conization, so that the initial cytological diagnosis could finally lead to an appropriate therapeutic approach.
In the quoted above project, there is also an organized system for telepathology that allows to manage histological cases from Italy: all slides are inserted in a special scanner that makes a digital image of every slide; the images are then saved in a local server accessible through Internet by a special website; in Italy some experts can access the website to see slides and render diagnoses.

All slides are prepared by two local technicians, who have been trained with a course held on the place by some POF’s members; after the course, the same technicians were also able to read pap smears. At present time, they managed by themselves negative cases and, by means of a digital camera connected to their microscope, they obtain selected images of all suspect or positive smears and again send these images through Internet for a definitive diagnosis rendered by Italian experts.

The seminar aims to be a practical demonstration of the feasibility of this remote diagnostics system if performed applying the necessary quality checks at every stage of the process: entrusting of diagnoses to qualified personnel, regular blindly revision by external experts of all cases reported as negative by the Zambian technicians and comparison of every positive cytological diagnosis with the subsequent histological diagnosis.

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**Session V**

**Cytopathology in Breast Pathology**

**Chairmen**

*M. Papotti (Torino), M. Truini (Genova)*

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**Breast cytology diagnostic role in the screening program**


UCO Citodiagnostics i E Histopatologia Azienda Ospedaliero Universitaria di Trieste; "UCO di Radiologia Azienda Ospedaliero Universitaria di Trieste; "**Agenzia Regionale Sanità Regione Friuli Venezia Giulia; "*** Dip. Matematica e Informatica Università di Trieste

All Italian regions activated during the last years screening programs for cancer prevention with different organizational models from region to region. An active breast mammography screening has started in Friuli Venezia Giulia since January of 2006 coordinated by the Regional Health Agency. Women aged between 50 and 69 are invited to take mammography every two years. During the first round (2006-2007) 152,388 aged between 50 and 69 are invited to take mammography screening has started in Friuli Venezia Giulia since January of 2006 coordinated by the Regional Health Agency. Women aged between 50 and 69 are invited to take mammography every two years. During the first round (2006-2007) 152,388 women were invited, 54.4% of them joined the program. Following are now mandatory for reporting of screening detected cases and this allows a standardization of the report as well as the continuous monitoring of the quality of benefits provided by breast units participating in the program.

Table I summarizes the distribution of the diagnostic categories and the following diagnostic procedures of the individual lesions undergoing FNA.

<table>
<thead>
<tr>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<td>4</td>
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<tr>
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<tr>
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<td>0</td>
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<td>+surgery</td>
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<td>13</td>
<td>92</td>
<td>33</td>
<td>34</td>
<td>191</td>
</tr>
</tbody>
</table>

B: Benign lesion; M: Malignant lesion

The Table above shows how the application of cytology has made it possible to select 88 nodules (66 women) with diagnosis C2 for which it was possible to avoid biopsy and that 187 nodules (170 patients) with a diagnosis C5 were directly referred for surgical therapy (mastectomy or quadrantectomy) without using of frozen sections. In 19 cases, also if in absence of conclusive cytological findings, the only correlation with the radiological findings made instrumental follow-up possible, which in all cases confirmed their benign nature. In cases with insufficient or inconclusive cytology (C1/C3/C4), total 31 cases, in the presence of suspicious radiological find-
ings Core Needle Biopsy has been used or the type of guidance has been changed (8 cases) and this has allowed to identify 17 cases of carcinoma and to avoid diagnostic surgery on 14 benign lesions. In only three cases stereotaxic biopsy was performed in presence of a benign cytology for the persistence of radiological suspects (all 3 cases were confirmed benign). In a small number of cases with malignant cytology (C5) Fine Needle Biopsy has been performed to characterize the lesion because of a possible neoadjuvant therapy (4 cases).

For each nodule the correlation with histological examination was performed or, for benign lesions not undergoing surgery, correlation with instrumental follow-up (mean 25 months) was done. No case was lost at follow-up and the correlation was possible even for those lesions with histology performed at other regional facilities due to the possibility offered by the Health Information System of Regione Friuli Venezia Giulia (INSIEL) which allows to share on the network all histological reports made by the Anatomical Pathology labs operating on the regional territory. The outcome of cytological correlation is summarized in Table II.

Thanks to the cyto-histological correlation, it was possible to calculate the quality indicators and compare them with the corresponding reference standards proposed in the guidelines for screening mammography summarized in Table III.

In the other lesions detected by screening, but not identifiable with ultrasonography (these are mostly isolated microcalcifications), it was necessary to refer patients for stereotactic VAB. The use of surgical diagnostic biopsy as first exam was limited to rare cases in which the stereotaxic guide was not technically possible or was refused by the patient (see Table IV).

The reliability of cytological diagnosis, confirmed by comparison of all quality indicators, has allowed the perfect integration of cytology in the multidisciplinary management of patients in the mammography screening program. This has provided a vital contribution in the diagnostic definition of breast lesions and has also made it possible to plan in most cases the surgical therapy of malignant lesions in a single step (without frozen sections) in addition to avoiding unnecessary biopsies on benign lesions, with obvious positive effects in terms of cost and effectiveness for the program.

References
1 Osservatorio epidemiologico nazionale screening (7° rapporto) 2008.

Liquid based Cytology in Breast Cytopathology
G. Simone
Cytopathology Unit. Cancer Institute “Giovanni Paolo II”, Bari, Italy

As well pointed out by Fernando Schmitt, the choice of using Liquid Based Cytology (LBC) lead to a cultural problem. In fact, many Pathologists, very used and experienced to use conventional smears, are unavailable to change their approach to Fine Needle Cytology (FNC) of the breast lesions.

Indeed, the two methods have not to be considered alternative but complementary, showing when compared, advantages and disadvantages.

Since 1990th LBC has been widely developed, gaining popularity in gynaecologic cytology and in the analysis of biological fluids, whereas its use in FNC is still limited. Therefore, there are few papers about the comparison between LBC with CSs in breast FNC, in which, a potential lower specificity of LBC was emphasized. However, it’s to note as in the paper of Biscotti et al., none C5 (malignant) case was reported being the low specificity due to benign lesions diagnosed as “atypical”. Bedard, in a study spanned on 28 years, also evidenced a slight lower specificity of LBC, but reported that when only

---

**Table II.**

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign Histology</td>
<td>1</td>
<td>4</td>
<td>19</td>
<td>8</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>Malignant Histology</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>25</td>
<td>191</td>
<td>224</td>
</tr>
<tr>
<td>Instrumental Follow up</td>
<td>7</td>
<td>88</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>107</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>92</td>
<td>33</td>
<td>34</td>
<td>191</td>
<td>363</td>
</tr>
</tbody>
</table>

*Instrumental follow up did not outline presence of malignant lesion.

**Table III.**

<table>
<thead>
<tr>
<th></th>
<th>Observed value</th>
<th>Reference NHS value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute Sensitivity</td>
<td>88.3%</td>
<td>&gt; 60%</td>
</tr>
<tr>
<td>Complete Sensitivity</td>
<td>97.8%</td>
<td>&gt; 80%</td>
</tr>
<tr>
<td>Complete Specificity</td>
<td>66.7%</td>
<td>&gt; 60%</td>
</tr>
<tr>
<td>Specificity for cases undergoing only biopsy</td>
<td>12.5%</td>
<td></td>
</tr>
<tr>
<td>Positive Predictive Value (C5)</td>
<td>100%</td>
<td>&gt; 95%</td>
</tr>
<tr>
<td>Positive Predictive Value (C4)</td>
<td>75.8%</td>
<td>70-80%</td>
</tr>
<tr>
<td>Positive Predictive Value (C3)</td>
<td>9.1%</td>
<td>&lt; 20%</td>
</tr>
<tr>
<td>False Positives Rate</td>
<td>0%</td>
<td>&lt; 5%</td>
</tr>
<tr>
<td>False Negatives Rate</td>
<td>0%</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Inadequates</td>
<td>3.6%</td>
<td>&lt; 25%</td>
</tr>
<tr>
<td>Inadequates Rate on Cancers</td>
<td>2.2%</td>
<td>&lt; 10%</td>
</tr>
<tr>
<td>Suspicious Cases (C4) Rate</td>
<td>18.5%</td>
<td>&lt; 20%</td>
</tr>
</tbody>
</table>

Meeting the imposed reference standards has been made possible by the direct and continuous participation of the pathologist in the cytologic sample’s collection. This has helped to keep the inadeguates number very low and to avoid false positives and negatives. Using the rapid cytology to immediately discuss with the radiologist about patient management in the same session provides great results for the patients.
the more recent 4 years are observed, there is not a significant difference in diagnostic accuracy. However there are morphological and practical aspects which render different LBC versus C:\(^4\): LBC presents a similar cellularity than CS, better nuclear detail, a lack of obscuring background and presence of monolayered arrangement with well preserved cells and architectural features. A problem for the LBC could derive from the lost of informative background, the fragmentation of epithelial cell clusters and the presence of rounded bipolar cells. From a practical point of view, the LBC consents, in clinical settings where FNC is not performed from the Pathologist or a skilled Radiologist or Clinician, to reduce inadequate samples. Moreover, LBC seems to be: a) easier and less time consuming method b) Reproducible, available for quality assurance protocols c) Usefull for immunocytochemical and molecular assay. The last issue is, in perspective, the most important. We remark as Tisserand et al.,\(^6\) demonstrated LBC be useful to also in preserving material for mRNA detection, also after 6 months at 4°C. and, looking to the target therapy with Herceptin, FNC on metastatic sites is a recommended tool to detect HER/2, by FISH. The detection of tumoral, prognostic-predictive factors is very important in the management of advanced breast cancer patients allowing to study biological change during cancer progression. Molecular data have to be correlated with morphological features and it is important to standardise both analitical and preanalytical phase of the assay, also in cytological samples, in order to their application in diagnosis, prognosis and target therapy. In this, for example, the sampling according to “direct to sample” or “split to the sample” appear to be more appropriate than the washing of the syringe after conventional sampling. Also according with the development of proteomic and genomic techniques, the Cytopathologist, who firstly approach cancer cells, has to be ready to give information and morphological support. In this issue we also report the results of 2 experiences: In first one, we compared data on prognostic and predictive factors obtained on 40 Core Biopsy specimens with corresponding tissue achieved after surgery and of 50 LBC-FNC of the breast also compared with corresponding surgical tissue: any significant differences was evidenced when Core Biopsies or LBC/FNCs were considered. Moreover we showed a preliminary study on FNC on metastatic lesion (30 cases) as an useful marker for a biological follow up. In this study, based on our previous experience, FNC showed to be able to find different subsets of patients with altered or conserved ER and/or HER-2/Neu status, during tumor progression. In conclusion LBC is an available tool for using in Breast FNC, particularly in reducing inadequate samples and to apply immunocytochemical and molecular assays. At least, the question is: Between LBC an CS, who seems be the best? The answer is that “Nobody is perfect and it depends from the context!”

References

Round table:
Can urinary cytology be improved with new technologies?

Use of a FISH assay to detect chromosomal abnormalities in urinary specimens

M. Paglierani, F. Castiglione, M. Pepi, M.R. Raspoilinni, G.L. Taddei
Department of Human Pathology and Oncology, University of Florence, Italy

Interphase fluorescence in situ hybridization (FISH) is a non-invasive assay detecting chromosomal abnormalities in urothelial cells of urine specimens. The multitarget UroVysion\textsuperscript{TM} (Vysis-Abbott, Abbott Laboratories. Abbott Park, IL) assay was initially approved by the US Food and Drug Administration (FDA) only for patients with a history of bladder cancer. In 2005 UroVysion\textsuperscript{TM} was granted FDA approval to be used as a bladder cancer screening tool in patients with gross or microscopic hematuria, but no previous history of bladder cancer. The probes mix consists of four fluorescently labeled DNA probes: three chromosome enumeration probes (CEP) to the pericentromeric regions of chromosomes 3 (SpectrumRed), 7 (SpectrumGreen) and 17 (SpectrumAqua) and one locus-specific identifier (LSI) to the 9p21 band (SpectrumGold) location of the p16 tumor suppressor gene. Diagnosis of recurrence and progression in the surveillance of bladder cancer is a challenging issue. At present, follow-up relies on the combined use of cystoscopy and conventional urinary cytology but the accuracy of both methods is low and interpretation of visible findings is operator-dependent. Cystoscopy is a highly sensitive technique, except in cases of flat malignancies such as pTis, but it is too much invasive. Cytology is a noninvasive technique, associated with high specificity, but it lacks sensitivity and produces high rates of suspicious cases mainly in case of low-grade (well-differentiated) tumors. Many studies have shown that UroVysion is more sensitive and equal or more specific than urinary cytology in diagnosis and monitoring of bladder cancer. The FISH test applied at the same thin layer slide used for cytological analysis is an excellent tool to resolve equivocal cytologic findings. The FISH
test is able to detect recurrences in the surveillance of bladder cancer before cystoscopically visible lesions can be identified. Furthermore recent studies also suggest that UroVysion may be employed in the diagnosis of upper urinary tract urothelial carcinoma and for determining therapy efficacy in patients treated with bacillus Calmette-Guerin therapy.

The FISH assay to detect chromosomal abnormalities in tumor cells is an objective tool of molecular cytology that has been shown to present advantages over routinely used urinary cytology. However, the method is both time and money-consuming, and reading and results interpretation must be entrusted to appropriate physician with extensive expertise.

Practical cytopathology: how to improve diagnosis and experience

D. Ientile (Palermo), F. Quarto (Castellamare di Stabia)

Practical cytology: like improving the diagnosis with the experience

F. Tallarigo

U.O.C. Anatomia Patologica e Citodiagnostica, ASP Crotone

In the course of approximately 150 history years the cytology has crossed several phases of development until our days, in which the possibility can be caught a glimpse to trace the entire genetic profile beginning from a single cell. The cytology is a branch of the doctor-biological science that studies the form, the structure and the organization of the cells that constitute the tissues in normal situations and under disease. Examinee to the microscope the cells that constitute the tissues of the human body is possible to find eventual alterations that they afford us to formulate a pathology or normality diagnosis.

The practical cytology is a discipline that is based or on the diagnostic cytology that on the cytological examination, the fusion of both affords to formulate and to improve the diagnosis. The diagnostic cytology is based on the observation of cells, deposited on the vetryn is normal or pathological, coming they from biological liquids and secretions, or from material abraded from the surface of a corporea or inhaled area with thin needle from a deep center, while the cytological examination is the morphologic analysis of cells captured from organs or tissue by means of it I use of different applicative methodologies.

Beginning from 40 years the practical cytology has quickly an important evolution, improving above all the methodical and preparation of samples, with a consequent improvement of the adequacy of the sample.

The diagnosis improves under investigation taking also requisitioned essential not negligible which: the clinical history of the patient (genetic ages, factors); the data of laboratory, comprehensive of eventual therapy and surgical participations progressi; modality of the withdrawal of sample cytological; methodical techniques and of laboratory used in the preparation of the prepared ones.

The cytological samples can be collected second various techniques: exfoliative cytology (es. liquid of deposit, urinary and exfoliative cytology of the oral cavity); abrasive (es. ulcers of superficial and deep organs).

The diagnostic preparation of prepared for cytological from biological liquids or of washing is a practical setting up many years ago, and generally it previews a preliminary enrichment of the champion through centrifugalization or filtration, therefore the warehouse of the cells on vetryn and then the fixation and coloration. The studies on the preparation of samples “in monolayer” or “thin layer “have afforded to remarkably deepen and to improve the classic techniques of separation of the cells from a liquid and the preparation of optimal champions for cytdiagnostic. The cytology in thin layer is a new approach for the preparation of the exfoliative cytology. This method affords the preparation of a thin film that theoretically could be read easy from the citopatologo, as the material turns out fixed in optimal way, the bottom of the prepared one turns out transparent, with remarkable reduction of the quota in blood, mucus and derid cellular.

Even if during the last few years this methodical one is applied in various centers also to the aspiration-cytology, its application is prevailing in the urinary cytology, surveying this of fundamental importance for the diagnosis and the follow-up of patients with neoplastic cells of the urinary tract. Such methodical cytological one, however, anticipates some limits which the necessity of reading an taken care of from an expert cytopatholog and frequent negativity of the examination in the forms to low degree of malignant neoplasm (papillary carcinomas of 1° degree). This last in fact they anticipate insufficient exfoliation, with the exception of the neoplasie of elevated degree more and above all atipie do not anticipate cytological. The urinary cytology is equipped of an elevated specificity but of a lowland sensibility, than varied to varying of the degree of differentiation of the neoplastic cells, while the cystoscopy is a procedure somewhat invasive that demands the collaboration of the patient. All this has carried during the last few years to the search of more reliable, less invasive and possibly quantitative tests diagnostic for the diagnosis and the follow-up of patients with carcinoma of the urinary tract. In these neoplastic forms, multiple genetic alterations (numerical and structural are identified) non-random. The survey of these specific chromosomal anomalies, with the urinary cytology, has been possible thanks to the application, to this examination, of the FISH (Hybridization In Fluorescent Situ). The FISH identifies the numerical anomalies of the chromosomes and of it characterizes the structural anomalies. In order to estimate tumor-associate chromosomal anomalies urothelial cells are used various probes (UroVysionTM, Vysis, Inc, Downers Grove L, etc.) marked with directed fluorescent substances towards the centromery of chromosomes 3, 7 and 17, and a specific probe for the locus 9p21.
To light of the present data in Literature that demonstrate to the correlation between some genetic anomalies and the degree of recurrence of the urothelial carcinoma, the FISH can play a decisive role in finding alterations of equips chromosomic with a urothelial cell before of the expression neplastic. Therefore, the presence of alterations in the urothelio not neoplasic evidenced with this technique (FISH) can help to evidence patients to high risk of recidivous. Recently the traditional cytology is integrated by ulterior methodical diagnostic, than they melt on the quantitative analysis of the DNA, which the cytometria to flow (automatic procedure, than measure il contenuto in DNA of the cells on samples of washing liquor urothelial cells) and QFIA (Quantitative Fluorescent Image Analysis) methodical this that uses a microscope to fluorescence controlled from a computer that automatically analyzes the nucleus of every cell.

From the methodological point of view, a methodical one that is to the base of the practical cytology and that in the course of the years it has quickly of the great changes is the technique of withdrawal of sample in the aspiration cytology with thin needle. Such technique has a fundamental role for the good resolution of surveying, also being relatively simple, goes executed with correctness in order to obtain the maximum amount of material in the best conditions than conservation. Based on the type of lesion, it is gone to use the needle characterized from some variable parameters as the external diameter and the length. In the sampling, for example, of thyroid nodules and concrete formations many vascular in order to diminish the entity of the hematic polution are of fundamental importance use it of very thin needles (25-27 gage).

Ulterior modality of cytological sampling is so said withdrawal of sample with needle without aspiration that found myself on the principle to make to go back the cells within the lume of the needle for capillarity. Such new sampling method is used above all if of the lymphonodes as not the traumatismo from aspiration cytology it habitually returns the morphology cellular of optimal quality, regarding the aspiration cytology methods. It is famous that the puncture of the concrete nodules can be carried out without some difficulty simply immobilizing the lesion between the fingers. The fine needle aspiration cytology of the deep masses it demands more complex sagacities. Fundamental for this purpose it is the aid of the techniques radiological. The radiological techniques of guide bring a substantial contribution to the cytology practical or by means of I use it of apparatuses of common use as the echography or by means of I use it of more sophisticated techniques (TAC).

Other diagnostic fields in which the cytological examination it plays a fundamental role are pathology of the salivari glands, that thyroid and, above all, the neoplastic pathologist of the ductal carcinomas of breast
The thyroid cytology from Fine-needle aspiration with (FNA) allows pre-operatory to define the nature of the thyroid lesions. Nodules of $\phi < 1$ cm. ecographycament but not clinical found can escape “tecnicamente” to the FNA. The cytology from aspiration with needle-thin is a technique employed for the first time in the diagnostic one of the thyroid nodules in 1948, and the maximum development of the concerning studies this diagnostic method is observed in the successive years for merit of Scandinavian authors. Currently it is a technique that in expert hands offers a remarkable diagnostic accuracy, with an elevated sensibility and specificity: he is ripetibile, of low cost and is insufficient invasive. For such characteristics in many centers it by now constitutes the first approach to the diagnostic one of clinical obvious the thyroid nodules that in the not endemic zones hit the 5-10% of the general population.

Important advantages in terms of sensibility and diagnostic effectiveness have reached with the FNA introduction assisted echo and TAC. Indeed the development of the ultrasonography techniques with the development of ecografiche probes with frequency of 7,5-10 MHz, has allowed to evidence nodulari lesions thyroid sub-centimetriche. This has afforded to observe clinical hidden the thyroid nodules.

The thyroid practical cytology has been improved in the time not only using of the ultrasonography radiological techniques that sure contribute in executing a good withdrawal of sample but the preparation is also important, Thanks to the advent of the biotechnologies are applied also in this defined field the methodical one “in liquid phase” or “on thin layer”. This methodical innovative partially automated one that more affords an improvement of the total quality of the examination, reaching a new one and elevated level than diagnostic accuracy being the disposed cells in an only layer on the vetrino, without emazie or inflammatory material, consequently allows to prepare of prepared cytological the much methodical luminosity and cleaned regarding the traditional one and to conserve a quota the same material that can be used for the application of methodical more sophisticated (es. immunocitochemical) or for molecular studies.

Regarding at last, the systematic study of mammary pathology by means of the cytological examination, this is undertaken by some ten of years in numerous characterized centers, however from relatively little time it has only found wide employment in the practical clinic. The first studies on the use of the fine-needle aspiration (FNA) go back to the first goal of 1900. In the second half of 1900 the study of the aspiration cytology in the diagnosis of the mammary neoplasie was only introduced in EUROPE. The role of thefine needle aspiration cytology of mammary is that to define the benign or malignant nature of a lesion. Moreover the cytopathologo needs of some data important before to formulate the diagnosis: age of the patient, the time of insurgence of the nodule, the clinical impression (with relative data to the factors of risk, progressi participations chirurcigi and eventual treatments in existence), the outcome of the mammographic examination and the precise center of the lesion.

In case of malignant lesion, in presence of tumors in phase above all advanced that they need of one chemioterapico treatment that precedes that surgical one, the cytological examination plays a role important because besides to define the nature of the lesion, it allows to establish those parameters prognostic (expression of hormonal receptors, and index of proliferation etc) that they orient chosen of the treatment.
Short course: Endometrial cytology

A.M. Buccoliero, G.L. Taddei
Department of Human Pathology and Oncology, University of Florence, Italy

Several diagnostic procedures are available to investigate the endometrium. Among these, endometrial cytology is the less utilized. The improvement of the diagnostic capacity of the endometrial cytology related to the introduction of the liquid-based method suggests to use this test in the endometrial diagnosis.

Contrary to cervico-vaginal cytology which has been precisely codified as far as both diagnostic criteria and reporting format, endometrial cytology has not been standardized. We are going to focus the diagnostic and reporting criteria in liquid-based endometrial cytology used at our Institution as result of the experience matured in eight years of utilize of liquid-based methodology in endometrial cytology.

Statement on specimen adequacy. Specimens are considered inadequate for diagnostic evaluation when they contain less than 6 epithelial endometrial cell clusters. Moreover, specimens are considered unsatisfactory for the diagnosis also when insufficient clinical information (i.e., age, menopausal state, menstrual state, hormonal therapy, risk factors, symptoms) are provided.

Diagnostic criteria. Diagnostic criteria are based on cytological and architectural evaluations and consider the epithelial and stromal endometrial cells and the background. Proliferative endometrium is characterized by the presence of three-dimensional cylindrical epithelial endometrial clusters. Cytoplasm is scant. Nuclei are isomorphic with finely granular chromatin. Nucleoli are small or absent. Cellular polarity is preserved. Stromal cells are abundant and spindle-shaped. Background is clean. Secretary endometrium shows wide three-dimensional cylindrical epithelial clusters. Bi-dimensional placards may be present in late secretory phase. Cytoplasm is clear and obvious. Nuclei are isomorphic with dispersed chromatin and small or absent nucleoli. Cellular polarity is preserved. Stromal cells are abundant and decidualized (wide cytoplasm, round nuclei, finely granulated chromatin, micronucleoli). Background is clean or, in late secretory phase moderately inflammatory. Endometrial atrophy is characterized by the presence of small cylindrical three-dimensional epithelial clusters. Epithelial clusters may appear swollen in cystic atrophy. Cytoplasm is scant. Nuclei are isomorphic with dense chromatin and small or absent nucleoli. Cellular polarity is preserved. Stromal cells are abundant and spindle-shaped. Background is clean. Multinucleated histiocytes are often recognizable. Hormonal administration determine endometrial morphological modifications mainly depending on the type of the administrated hormone, the dosage, the regimen (combined or sequential estrogen-progestin administration) the duration of the administration and, in fertile women, the menstrual phase in which the hormone is administrated. The estrogens, when unopposed by the progestins, produce a proliferative input on the endometrium determining a possible hyperplastic and even neoplastic progression. On the contrary, the progestins are responsible for proliferation arrest, glandular secretion and decidualization of the stromal cells. The prolonged progesterone treatment induce progressive arrest of the secretion and glandular atrophy. Cytological features in endometrial specimens reflect these hormonal induced modifications. Endometrial Hyperplasia appears in cytological samples in form of numerous wide three-dimensional epithelial endometrial clusters with variable cellular crowding and architectural disorder. In typical endometrial hyperplasia the cytoplasm is commonly scant and the nuclei are isomorphic with finely granular chromatin and small or absent nucleoli nucleoli. In atypical endometrial hyperplasia the cytoplasm becomes evident and the nuclei may show a moderate-grade of pleomorphism. Spindle shaped stromal cells are abundant in typical hyperplasia while they are less represented in atypical hyperplasia. The background may enclose inflammatory cells. Main diagnostic criteria for endometrial carcinoma are:

1. Architectural (loss of polarity, papillary cell clusters, dysmorphic cells);
2. Cellular (high nucleo/cytoplasmatic ratio, anisonucleosis and poikilonecrosis, coarse and/or margined chromat, nucleolar prominence, nuclear membrane incisures, cell cannibalism);
3. Background (scarcity of stromal cells, necrosis). Cell cannibalism is observed more often in poorly differentiated tumors.

Specimens obtained from patient affected by serous carcinomas are hypercellular (small cellular clusters with inconspicuous cellular crowding, single cells, bare nuclei). Psammoma bodies are sometimes seen.

Endometrial cytology is efficacious diagnostic procedure. It could be applied alone or in association with other diagnostic procedure improving their diagnostic accuracy.
Free presentations

The indeterminate thyroid fine-needle cytology: 2-year experience from a single academic center

V. Ascoli, D. Bosco, G. Caruso, G. Deriu, C. Taffon, L. Marinelli, D. De Mattia, L. Grillo, F. Nardi
Laboratorio di Citodiagnostica, Dipartimento di Medicina Sperimentale, Università La Sapienza, Roma

Introduction. Both SIAPEC and British Thyroid Association have developed a 5-tier reporting system for thyroid fine-needle cytology (FNC), which include the “indeterminate” category (THY-3). In such cases, FNC is not able to give a final diagnosis and patients are often referred to surgery, considering the approximately 20% rate of malignancy.

Methods. In our laboratory, the 5-tier reporting system has been used for 5680 thyroid aspirates in the last 28 months (2007-june 2009). We assessed the distribution of aspirates by the 5 diagnostic categories, and then we focused on THY-3 cases by evaluating the proportion of resected cases in our hospital facilities and the histological diagnosis.

Results. A total of 239 cases (4.2%) were interpreted as THY-3, of which 101 (42.2%) had surgical follow-up. The surgical yield of malignancy was 22.8% (18 papillary carcinoma, 1 follicular carcinoma, 3 Hürthle cell carcinoma, 1 metastatic renal cell carcinoma); 22.8% were adenomas (15 follicular and 8 Hürthle cell adenomas) and the remaining 54.4% were negative (43 nodular hyperplasia, 9 Hashimoto’s thyroiditis, 3 chronic thyroiditis). Four occult papillary carcinoma were identified as incidental finding either in the controlateral lobe (3 hyperplasia) or in the omolateral lobe/additional nodule (1 adenoma).

Conclusions. Although the follow-up of the THY-3 category is not available for all cases, our results are in agreement with literature concerning malignancy rate and prevalence of occult thyroid carcinoma, and indicate that more than a half of the THY-3 cases undergoing surgery could potentially benefit of FNC repeat instead of unnecessary surgery. It has to be better understood (i) how to determine by cytology which fraction of THY-3 patients need surgery versus FNC follow-up, and (ii) the potential utility of a 6-tier diagnostic scheme for reporting thyroid FNC, as proposed by the National Cancer Institute (Diagn Cytopathol 2008 Jun;36:425-37).

<table>
<thead>
<tr>
<th>FNA-category</th>
<th>Number of aspirates (%)</th>
</tr>
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<tbody>
<tr>
<td>Thy-1</td>
<td>2224 (39,1)</td>
</tr>
<tr>
<td>Thy-2</td>
<td>3075 (54,1)</td>
</tr>
<tr>
<td>Thy-3</td>
<td>239 (4,2)</td>
</tr>
<tr>
<td>Thy-4</td>
<td>138 (2,4)</td>
</tr>
<tr>
<td>Thy-5</td>
<td>4 (0,07)</td>
</tr>
<tr>
<td>Tot.</td>
<td>5680</td>
</tr>
</tbody>
</table>

* Histology in 101 cases: (a) Benign lesions n = 55, (b) Adenomas n = 23, (c) Malignancy n = 23.

Fine-needle aspiration biopsy (FNAB) and core needle biopsy (CNB) of renal masses in adults. Retrospective analysis on 124 cases

A. Barreca, D. Pacchioni, G. Accinelli, I. Garetto, A. Veltri, E. Bollito, M. Papotti, G. Bussolati
Department of Biomedical Sciences and Human Oncology, University of Turin, Italy; Institute of Radiology, University of Turin, Italy; Division of Pathology, Department of Clinical and Biological Sciences, San Luigi Hospital, Orbassano, Italy

Introduction. FNAB of the kidney has a well-defined role in the diagnosis and treatment of renal masses. In fact due to the new therapeutic approaches (nephron sparing surgery and percutaneous thermal ablation) a pre-treatment accurate pathological diagnosis of renal masses should be obtained. The purpose of our study was to report the diagnostic yield of 124 imaging-guided biopsies of renal masses.

Material and methods. We retrospectively reviewed 124 percutaneous needle biopsies (122 US-guided and 2 TC-guided) of renal masses (10–150 mm in diameter, mean 33,7) performed from June 2002 to March 2009. The radiological aspect of the lesion was solid in 107 cases, cystic in 8 and mixed in 9. 25 patients underwent FNAB (21-22 G, cytologically examined), 36 CNB (18 G or larger, histologically examined), 63 both in sequence. Two expert pathologists blindly reviewed cytological and histological samples. In 61 cases (49%) immunohistochemistry was used to establish or confirm the diagnosis. Pathological diagnosis were compared with clinical outcome in 114 cases (34 surgical resection, 46 radiofrequency ablation, 2 radiofrequency ablation and surgical resection, 30 watchful waiting, 2 medical treatment).

Results. 124 patients (79 men, 45 women) ranging in age from 31 years to 85 years were considered. An adequate sample size was obtained in 110 (89%) of 124 renal masses and led to a definitive diagnosis in 106 (85%) of the 124 cases. They included 80 (75%) malignant lesions constituted by 77 renal cell carcinoma (47 clear cells, 20 papillary, 4 chromophobe and 6 not determinate), 2 lymphomas and one indifferentiated carcinoma. The diagnosis of a benign tumour was made in 26 (25%) of 106 biopsies and included 16 oncocytomas, 2 angiomylipomas, 2 leiomyomas, 3 benign cysts, 2 metanephric adenomas and 1 renal parenchyma with sclerosis. The histological concordance of biopsies with definitive diagnosis and surgical specimens was excellent.

Conclusion. Imaging-guided biopsy of renal masses was diagnostic in 106 (85%) of 124 cases. The preoperative diagnosis decreases the rate of unnecessary surgery and can assist the clinician with treatment decision making.

References
Cytology of eosinophilic dysplasia of the cervix uteri

Department of Cytomorphology, Section of Pathology, University of Cagliari, Italy; † Department of Pathology, Central Hospital Bolzano, Italy; ** Explora, Research and Statistical Analysis, Padova, Italy

Background. Eosinophilic dysplasia (ED) of the cervix uteri is a particular type of dysplasia that retains metaplastic features and is characterized by cells with relatively large eosinophilic cytoplasm. The aim of this study was to investigate the cytologic features of ED.

Materials and methods. Histological samples from 82 women with the diagnosis of ED were collected from the archive of the Department of Pathology of the Central Hospital of Bolzano. All women had had at least one pap-test within the previous year. Immunohistochemical analysis for p16 reactivity was performed in order to confirm the dysplasia. Control specimens included 31 biopsies of high-grade Cervical Intraepithelial Neoplasia (CIN 2-3) of usual type with the respective pap-test.

Results. After revision of the histological samples, an ED was confirmed in 66 out of 82 cases (80.5%). P16 reactivity was detected in all 66 cases of ED. In 58 out of 66 cases (87.9%), the ED was associated with a CIN of usual type. The revision of the previous pap-tests revealed in all cases Atypical Squamous Cells (ASC) or Squamous Intraepithelial Lesion (SIL). In 56 (84.8%) of these cases, dysplastic cells with hypochromic nuclei were found associated with a conventional SIL. In 60 out of the 66 cases (90.9%), the SIL showed metaplastic features. Both features were concomitantly present in 51 out of 66 (77.3%) cases. Dysplastic cells with hypochromic nuclei or metaplastic features were present in 7 out of 31 (22.6%) and 4 out of 31 (12.9%) of the control SILs respectively.

Conclusions. Eosinophilic dysplasia of the cervix uteri is mostly associated with CIN of usual type. A HSIL with hypochromic nuclei or metaplastic features is often found in the pap-test previous to the histological diagnosis of ED and may represent the cytologic correlate of this particular type of dysplasia.

The utility of uCyt+ in the detection and surveillance of transition cell cancer of the bladder (UC) and its application in differentiating follow-up schemes

A. Bernardi, E. Berno*, F. Fop**, M. Gussio, G. Maffei, N. Martinetti, F. Morabito***, P. Lovadina, E. Berardengo

Introduction. The aim of this study was to evaluate whether the uCyt+ test improves the detection of urothelial carcinoma (UC) and also the surveillance during follow-up, and, if it is a valid parameter in the choice of follow-up schemes.

Materials and methods. A four-year study was conducted concerning 712 patients (pz) divided into three different groups: 198 pz at their first detection (Group 1), 317 pz (Grupp 2) with previous UC (Grade 1 according to WHO in 163 pz, Grade 2 in 58 pz, Grade 3 in 59 pz, and in 37 pz the grade was not available), and 197 pz with previous prostate problems (Gruppo 3). All three groups were monitored according to the standard detection and follow up protocols and the uCyt+ immunofluorescence test (a total of 1522 tests) on voided urine samples.

Results. Groups 1 and 2 showed a strong association (p < 0.0001) of their uCyt+ and cytology data with cystoscopy results, while Group 3 did not evidence statistical significant data.

The statistical analysis of Sensitivity, Specificity, Positive Predictive Value (P.P.V.), Negative Predictive Value (N.P.V.), Cohen K and Odd Ratio (OR) of the uCyt+ and Cytology exams of groups 1 and 2 are illustrated in the following Table.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2 Overall</th>
<th>Group 2 G1</th>
<th>Group 2 G2</th>
<th>Group 2 G3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>uCyt+</td>
<td>Cyt.</td>
<td>uCyt+</td>
<td>Cyt.</td>
<td>uCyt+</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>71%</td>
<td>64%</td>
<td>83%</td>
<td>50%</td>
<td>86%</td>
</tr>
<tr>
<td>Specificity</td>
<td>93%</td>
<td>85%</td>
<td>91%</td>
<td>89%</td>
<td>92%</td>
</tr>
<tr>
<td>P.P.V.</td>
<td>48%</td>
<td>45%</td>
<td>57%</td>
<td>41%</td>
<td>51%</td>
</tr>
<tr>
<td>N.P.V.</td>
<td>98%</td>
<td>97%</td>
<td>97%</td>
<td>92%</td>
<td>98%</td>
</tr>
<tr>
<td>Cohen K</td>
<td>53%</td>
<td>48%</td>
<td>62%</td>
<td>39%</td>
<td>95%</td>
</tr>
<tr>
<td>OR</td>
<td>39%</td>
<td>25%</td>
<td>48%</td>
<td>8%</td>
<td>58%</td>
</tr>
</tbody>
</table>

Conclusions. The uCyt+ test performed simultaneously with cytological and cystoscopy controls improves the detection and surveillance of UC and the uCyt+ test helps identifying patients that need to be treated with different follow up protocols.
Development of a diagnostic EpCAM test

A. Böcking, I. Seitz

Institut für Cytopathologie, Universitätssklinikum Düsseldorf; Fresenius Biotech GmbH, Größefingen

Aims. The epithelial cell adhesion molecule (EpCAM) is expressed on the vast majority of epithelial tumors (carcinomas). As the lining of the peritoneal cavity consists of mesothelial cells that do not express EpCAM on their surface, EpCAM is an interesting antigen for specific targeting of epithelial tumor cells within the peritoneal cavity. For EpCAM-directed tumor therapeutics in malignant ascites a routine-compatible diagnostic test for detection of EpCAM-positive tumor cells in ascites fluid has been developed.

Methods and results. The EpCAM test is an immunocytochemical assay using cytopsin preparations of 2.5x10^4 ascites cells per spot. Following fixation, inhibition of endogenous peroxidase activity, and permeabilization EpCAM is labeled using the EpCAM-specific monoclonal antibody Ber-EP4 or HEA125. Antibody binding is visualized using the avidin-biotin complex method. The enzymatic conversion of the substrate 3-amino-9-ethylcarbazole results in the formation of a red-brown colored reaction product at the antigen site. Cells are counterstained with Mayer’s hemalaun and coverslipped. EpCAM-positive tumor cells are evaluated using a light microscope. The assay was validated and showed EpCAM specificity demonstrated by staining of EpCAM-positive and EpCAM-negative cell lines. The limit of detection was 8 EpCAM-positive in a total of 2.5x10^6 cells. Functionality of the assay was shown using native ascites samples from patients with epithelial tumors.

Conclusion. This diagnostic EpCAM test is a simple, robust and validated method for quantitative detection of EpCAM-positive tumor cells in serous effusions such as ascites fluid. Therefore, it allows therapeutic decisions for treatment of malignant ascites patients with drugs directed against EpCAM.

Squamous carcinoma of the lung metastatic to the breast

M. Bonzanini, P. Dalla Palma

Department of Surgical Pathology, S. Chiara Hospital, Trento, Italy

Clinical history. A 64-years-old woman, without any relevant prior clinical history, revealed at the screening mammography a nodule in the right breast, 10 millimetres in diameter, with malignancy features. The cytological smears showed irregular sheets of epithelial cells with pleomorphic, hyperchromatic nuclei and scanty cyanophilic cytoplasm. There were also cells with abundant eosinophilic cytoplasm and hyperchromatic nucleus. A diagnosis of “Malignant cells with features of high breast grade carcinoma, probably metastatic. However a metastatic squamous carcinoma can not be excluded” was performed. The clinicians have been in doubt about the last hypothesis. A X-ray of the thorax, one month later, revealed a hypo-diaphanous image in the medium-basal right region. A diagnosis of pneumonia was performed. Ten 10 days after, regardless the therapy, a slight improvement of the lesion with pleural effusion at the control X-ray was found. The bronchoscopy showed a stenosis of the right inferior bronchial tube. The bronchial aspiration and biopsy showed a squamous carcinoma. The total body CT scan revealed a solid mass, 5 cm of diameter, in the right inferior bronchial lobe tight to the right atrium and near to the oesophagus.

The patient were treated with chemotherapy. In the following months the CT scan showed a reduction of the pulmonary mass. At the mammography no increase of the nodule was documented. One year after, cutaneous and hepatic metastases were found. The patient died one year later.

Conclusions. Breast metastases are very rare accounting about 0.5-3% of patients with extra-mammary malignant neoplasm. In the 70% of cases the primitive neoplasm is known and the cytological smear is useful to confirm the diagnosis. Most rarely breast metastases represent the first sign of the neoplastic disease and often simulate clinically and cytotologically a primitive neoplasm. Of the tumours metastasizing to the breast, one of the most frequent is the small cell carcinoma of the lung. Squamous carcinoma metastasizing to the breast are described deriving from oesophagus, oral cavity, pharynx, cervix, vulva and lung. Some differential diagnoses should be considered when we observe malignant squamous cells in a breast FNA: squamous and adenosquamous metastatic carcinoma, low grade adenosquamous carcinoma and, finally, metastatic carcinoma. Our case confirms the importance of the contribution of breast cytology for the correct management of rare lesions too, avoiding, when possible, unnecessary surgical intervention.

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Braf mutations in thyroid fna samples with features indeterminate/suspicious for papillary thyroid carcinoma. A study of 23 cases with histological correlation

M. Bonzanini, S. Girlando, M. Barbareschi, L. Cuorvo, L. Morelli, P. Dalla Palma

Department of Surgical Pathology, S. Chiara Hospital, Trento, Italy

Introduction. Papillary carcinoma (PTC), the most common thyroid malignancy, frequently carries BRAF, RET/PTC, or RAS mutations. These mutually exclusive somatic mutations are found in more than 70% of papillary carcinomas, and some of them are associated with more aggressive tumour behaviour. BRAF belongs to the family of RAF proteins (ARAF, BRAF, CRAF), which are intracellular effectors of the MAPK signalling cascade. To date, multiple studies have confirmed that BRAF mutation is the most common event in sporadic adult papillary carcinomas and occurs in approximately 45% of all cases. Virtually all mutations found in these tumors are V600E.

Several studies have demonstrated the feasibility of detecting BRAF mutations in thyroid FNA samples and have shown that this may improve the cytological FNA diagnosis. Positive predictive value approaches 100% and a positive molecular test can be of high diagnostic value in indeterminate or atypical FNA.
Methods. We analyzed 20 indeterminate/suspicious (I/S) thyroid FNA samples with available histological examination. For all cases DNA was extracted from previously stained cytological samples and analyzed using the ABPrism 310 Genetic Analyser.

Results. BRAF mutation were seen in 10 cases. Histologically, all cases with BRAF mutation proved to be PTC, while among the 10 cases with wild type BRAF, 4 were PTC, 4 were follicular variant of PTC and 2 were nodular hyperplasia.

Conclusions. FNA of the thyroid plays a very important role in guiding the clinical management of the patient. However in some patients a definitive conclusions can not be reached and these cases are classified as I or S. These diagnostic categories constitute an average of 20-30% of cases in reported series, and 20-30% of them are proven to be malignant at histological examination. Recent advances in molecular genetics of thyroid cancer can be of help for a more accurate definition of these equivocal FNA samples. In our cases BRAF mutation was always associated with histologically proven PTC, while the benign lesions were all BRAF wild type, confirming the high PPV of the molecular test. Noteworthy, BRAF mutation was detected in 55% of PTC with I/S FNA.

Our data, in keeping with the results of retrospective and prospective studies, support the value of the analysis of Fna mutation in the management of the thyroid lesions with equivo-cal FNA.

References

Usefulness of P16INK4a in cervical cytology
C. Carnovale-Scalzo, M. Agosta, E. Loreti
Laboratory of Cytology, Machiavelli Medical House USI Group, Rome, Italy.

Introduction. The aim of our study was to evaluate the usefulness of P16INK4a immunostaining related to the cytological diagnosis in liquid based cervical cytology samples (ThinPrep Pap Test, Cytac).

Methods. The samples were selected from the archive of the cytology laboratory of MMH-USI in Rome. The research was carried out on a total of 233 samples from women (age range 18-69 years) and divided in the following diagnostic categories: 36 NILM, 38 ASCUS, 115 LSIL, 21 ASCH, 21 HSIL, 2 squamous cell ca. Slides were stained according to Pananicolau method, and cytological diagnosis was performed using the Bethesda 2001 terminology system. Multiple slides for P16INK4a immunocytochemical staining were processed from the residual material in the vial, following the CIINtec Cyto- kit procedure.

Results. There is a substantial correlation between P16INK4a immunostaining and cytological diagnosis. Number of samples with an overexpression of P16INK4a protein gradually increase with the severity of the injury. In negative cases (NILM n = 36), the immunocytochemical reaction was always negative. For ASCUS category, P16INK4a was positive in 26.3% of cases, for LSIL in 50.4%, for ASCH, HSIL and squamous cell carcinoma P16INK4a was positive in 100% of cases.

Conclusions. LSIL is the core cytological category in diagnostic and clinical survey because there is either a percentage of probability of disease to progress to severe lesions or that viral infection regresses, depending on the infecting viral type (HR-HPV) and its level of integration into the DNA of the cell.
Overexpression of p16 protein in LSIL appears to be a useful biomarker of disease progression, and it allows to restrict the number of low grade lesions patients (about to 50% cases) who should be submitted to more accurate follow up.

Common albeit remarkable cytologic features in thyroid tumours.

Description of two cases

G.P. Casadei
Ospedale Maggiori C.A. Pizzardi, Dipartimento di Anatomia Patologica, Bologna

Introduction. The common cytologic features of neoplastic thyroid lesions in some conditions may be puzzling or challenging and needs a careful diagnostic procedure. Two cases are reported that compellingly support this issue and illustrate diagnostic considerations in aspiration cytology, one related to the finding of intranuclear inclusions and the other of papillary features in thyroid fine needle aspiration (FNA).

Case report 1. The patient is a 47-years-old woman who came to the attention of the physician for a nodule appeared in the superior right lobe of the thyroid. Routine blood investigation revealed she was euthyroid with negative antibodies and with a little increase of calcitonin. The nodule measured 11 millimeter in diameter, echographically it was hypoechoic and solid. The FNA smears showed an abundant, monomorphic cellularity, with loose aggregates: the cytoplasm of the cells was large and nuclei were round, monomorphic, bland with rare incisures. Quite numerous were the nuclear inclusions. The background was bloody: no colloid, nor macrophages were present. The cytologic findings were considered consistent with a papillary carcinoma. A total thyroidectomy was performed. The histologic features revealed a well-circumscribed tumor organized in solid nests and cords bordered by hyaline fibrovascular stroma: the cells were large, polygonal with large eosinophilic cytoplasm arranged in orthogonal palisade. The nuclei had frequent incisures and inclusions. The cells showed a diffuse immunoreactivity for Ki-67. The pathologic diagnosis was hyalinising trabecular adenoma.

Conclusions. Even if the presence of intranuclear inclusions is a useful diagnostic criteria and hardly searched for the diagnosis of papillary papillary carcinoma, they are not pathognomonic of it. They have been described in other pathologic conditions of the thyroid, like in Hurthle cell neoplasia or in metastatic renal cell carcinoma. They are described in hyalinizing trabecular adenoma too. Cytologic diagnosis of papillary carcinoma rests on a series of cytologic features like monolayered, solid or papillary cellular sheets, nuclear incisures and inclusions, multinucleated cells, thick colloid. Most of these features should be present before to perform diagnosis of papillary carcinoma. Cytological diagnosis of trabecular adenoma might be suspected when large pale cells with nuclear inclusions are radially disposed around metachromatic material in a background without colloid, multinucleated cells, papillary and follicular structures. A surgical excision should be anyway recommended in the presence of such a cellular smear.

Case report 2. A 66-year-old man was referred to our hospital in 2005 for the appearance of a mass in the left lower neck since a couple of months. The lump measured 2 cm and at ultrasound (US) it was strongly hypoechoic, not homogenous with irregular borders. A surgeon quickly performed a US-guided FNA and a core biopsy. The cytological samples revealed strands and solid aggregates of cells with round-oval nuclei with frequent incisures and rare cytoplasmic nuclear inclusions. The microscopic examination of the biopsied tissue sections showed as well a carcinoma with papillary architectural pattern, psammoma bodies and nuclear pseudo-inclusions. At immunostains the cells were positive for TTF-1: the final diagnosis was metastatic papillary carcinoma of the thyroid. Staging of thyroid tumor led to the evidence at CT of a nodule in the right apical inferior pulmonary lobe: at this point steps were taken to perform bronchoscopy with transbronchial lung biopsy, FNA and bronchial washing. The FNA smears showed a bloody background with the presence of neoplastic cells in small pseudopapillary aggregates with frequent nuclear inclusions. A transbronchial biopsy was performed: the histological findings were consistent with a well differentiated adenocarcinoma with papillary features, microcalcifications and rare nuclear pseudo-inclusions. The cells were immunoreactive for TTF-1 and CEA, and negative for Thyroglobulin (TG) and vimentin. A diagnosis of a adenocarcinoma of the lung was made, most probably primitive. A bronchial lavage was positive too for a non-small cell carcinoma. The histological sections of the metastatic lymph node carcinoma were reviewed and showed faint immunoreactivity for TG, but not unequivocally: CEA immunostain was negative. The original diagnosis of metastatic papillary carcinoma of the thyroid was anyway favored. Finally a 8 millimeter left thyroid nodule was found at US and a FNA showed few neoplastic cells coherent with papillary carcinoma. Radical dissection of the thyroid with bilateral cervical nodes confirmed a well differentiated papillary thyroid carcinoma with omolateral lymph nodes metastases. Surgical resection of the upper and medium pulmonary lobes was performed: microscopic examination of the resected tissue revealed a well differentiated adenocarcinoma of the lung with papillary features. The patient died 2 years after the first diagnosis with diffuse bone metastases of pulmonary adenocarcinoma.

Conclusions. The coexistence of papillary thyroid carcinoma and adenocarcinoma of the lung with papillary features may give rise to problematic situations when the first diagnostic step is a FNA cytological diagnosis of a metastatic cervical node. In the reported case, the morphologic features of the two tumours were similar and out of TG, no immunostains was useful to define the primary origin of the metastatic neoplasia. The application of immunostains in cytologic smears is quite useful, but restricted since generally the scarce number of specimens reduces the possibility in using the different available markers. In such situations the reliability of using FNA to distinguish thyroid and pulmonary papillary tumors in a metastatic setting is questionable and a precise clinico-pathological correlation is quite necessary and paramount and as in our case, in spite of the location of the nodules could suggest the presence of two distinct tumours, a biopptic procedure is requested in order to have a histologic confirm to the diagnosis.

References

Kras mutation analysis of fine needle aspirate under eus guidance as a supplement of the cytopathology for the diagnosis of pancreatic adenocarcinoma

L. Daniele, D. Pacchioni, S. Mariani, M. Bruno*, P. Carucci*, C. De Angelis*, G. Bussolati
Department of Biomedical Sciences and Human Oncology, University of Turin, Italy; *Department of Gastro-Hepatology, Molinette Hospital, Turin, Italy

Introduction. Cytology from aspirates obtained by endoscopic ultrasound-fine needle aspiration (EUS-FNA) is the most precise single technique for the diagnosis of pancreatic adenocarcinoma. However the accuracy of this technique is suboptimal, since the differential diagnosis between pancreatic adenocarcinoma and pseudotumoral forms, such as chronic pancreatitis, remains difficult. Mutational activation of the Kras oncogene is almost universally present in pancreatic cancer tissue. We therefore investigated if analysis for mutant Kras gene in the EUS-FNA aspirates supplements cytopathology for the diagnosis of pancreatic adenocarcinoma.

Methods. EUS-FNA cytologic specimens obtained from 24 patients with pancreatic masses were analyzed for the presence of Kras mutation on codon 12 and 13 using restriction endonuclease-mediated selective PCR (REMS-PCR) and confirmed by direct sequencing. Mutational analysis was performed after quality and quantity controls of the DNA extracted. Final diagnoses were obtained on EUS-FNAB analysis and/or on surgical pathology; 17 patients had adenocarcinoma and 7 had benign lesions.

Results. Specific Kras point mutations were detected by REMS-PCR in 16/17 adenocarcinomas and in 2/7 cases without a cytological diagnosis of adenocarcinoma. Direct sequencing of Kras was performed in 12/17 adenocarcinomas and in 2/7 cases of pancreatic benign lesions due to the good amount of the DNA extracted and confirmed the presence of a specific mutation in 9/12 adenocarcinomas and in 1/2 cases of chronic pancreatitis mutated at the REMS-PCR analysis respectively.

Conclusions. Analysis for the presence of mutant Kras gene is able to supplement conventional cytopathology for the diagnosis of a pancreatic adenocarcinoma. Among patients with negative cytopathology the presence of Kras mutation could represent a pancreatic cancer while the absence of Kras mutation seems to increase the possibility of benign lesions. REMS-PCR technique seems to be more sensible than the direct sequencing of the Kras gene.

Pre-operative cytological diagnosis of stromal tumours on EUS-FNA samples

Department of Biomedical Sciences and Oncology University of Turin, Italy; *Department of Gastrohepatology, Molinette Hospital, University of Turin, Italy; **Center for Experimental Research and Medical Studies, University of Turin, Italy

Introduction. EUS-FNA has become standard practice in preoperative evaluation of mesenchimal subepithelial neoplasms of the gastrointestinal tract since it allows a clear identification of the submucosal origin and a sampling from the deeper tissue layers. Particularly, the importance of a pre-operative diagnosis of GIST lays in a proper labelling of the disease, (GIST versus other mesenchinal neoplasm, such as leymiomias or schwannomas), in the evaluation of its malignant potential and in the study of c-kit overexpression and mutation, involving a possible positive response to Imatinib. We present our 6 years experience in EUS-FNA guided mesenchmal lesions sampling and our efforts to make the best diagnosis even on a very limited amount of material in order to reach a detailed response useful for a correct surgical management of the patient.

Methods. From 2003 and 2008 we examined 27 submucosal neoplasms cases out of 600 EUS-FNAs. In all cases an on-site cytopathologist was present in order to: a) assess the adequacy and sufficiency of the aspired material; b) gather enough material for the cell block, and thus for the immunocytochemical/molecular biology studies.

Results. We had a mean of 6 smears and 2.7 cell block for each case. A cytopathological diagnosis of GIST was achieved in 14/27 (53.8%), in two case was made a diagnosis of leymiomia (7.7%), one diagnosis of PNET and two of synovial sarcoma were assessed (3.8%); in the remaining 8/27 cases (30.8%) we could not go further than a diagnosis of “stromal spindle-cell lesion”. Immunocytochemical markers employed were CD117 (23/27 cases), S100 and SMA (both studied in 20/27 cases), CD34 (19/27 cases) and AE1/AE3 (8/27 cases). Ki67 was studied in 17/27 cases. In-depth ICC examination was required to asses the diagnosis of PNET and synovial sarcoma. In 5 cases mutational status was also performed: two cases presented mutations.

Conclusions. On-site evaluation and triage of the material is a critical point in improving the accuracy of the cytological diagnosis of subepithelial mesenchimal neoplasms of GI tract.

Fine needle aspiration cytology of salivary glands lesions: cytologic-histological correlations of 210 cases

A.L. Delazer, M.D. Beccati, G. Binotti, C. Buriani, A. Carantoni, C. Cavicchi, S. Immovilli, I. Nenci*
Diagnostic Cytopathology, Sant’Anna University Hospital, Ferrara, Italy; *Morbid Anatomy, Surgical Pathology and Cytopathology, Ferrara University, Italy

Introduction. Identifying malignancy can have a significant impact on the management of salivary gland tumors. We review our experience with fine needle aspiration cytology (FNAC) and investigate 210 patients who had undergone preoperative FNAC of salivary glands and had been diagnosed by postoperative histopathological examination. We compare the findings of preoperative FNAC with their histopathological types in salivary glands tumors and discuss the results.

Methods. Retrospective study of 210 patients who had salivary glands lesions and were submitted to FNAC and surgical treatment.

Results. The accuracy of FNAC was 94.02, with a sensitivity of 86.96% and a specificity of 95.12%. The rates of agreement in the diagnosis of pleomorphic adenoma, Warthin tumor and basal cell adenoma were 95.45%, 94.73%, and 60.00% respectively.

Conclusions. It was shown that FNAC, as diagnostic method, can be useful for preoperative evaluation and surgical planning.
Utility of flow cytometry immunophenotyping in fine-needle aspirate cytologic diagnosis of non-Hodgkin lymphoma

Flow Cytometry Unit, Molinette Hospital, Turin; * Laboratory of Pathology, Molinette Hospital, Turin; ** Institute of Diagnostic and Interventional Radiology, University of Turin; *** Department of Biomedical Sciences and Human Oncology, University of Turin; **** Department of Clinical and Biological Science, University of Turin at San Luigi Hospital, Orbassano, Turin, Italy

Introduction. Flow cytometry (FC) immunophenotyping of fine-needle aspiration (FNA) has been reported to be useful in the diagnosis of non-Hodgkin lymphomas (NHLL). Moreover, it seems to be especially suitable for cytology specimens as it requires only a small sample, it is also suitable for cells already in suspension and has a rapid turnaround time.

Materials and methods. The authors reviewed their 5-year experience to assess the ability that FC has in improving the diagnostic capacity of cytomorphology in the diagnosis and subclassification of NHL according to the WHO classification. FC was performed on 252 FNA specimens. 123 cases of NHL (89 primary and 34 recurrent lymphomas). The FC immunophenotyping included CD3, CD4, CD8, CD10, CD19, CD20, CD45, and antibodies combinations in the screening panel and additional panels for B or T lineage in the presence of positivity for lymphoma after the screening. Aberrant staining intensity for T-cell markers was considered suggestive of T-LNH. In select cases, B-cell clonality was determined by the quantification chains on CD10 or CD5-positive B cells, on expression levels of CD19 or CD20 and based upon altered forward scatter. The other 129 FNA cases included benign/reactive process, metastatic carcinoma and melanoma, acute leukemia, myeloma, Hodgkin lymphoma, thyroiditis.

Results. An immunologic diagnosis was obtained by FC in 90% (111/123) of cases identified as NHL. FC was able to improve the total number of NHL detected in 8 cases where cytomorphology had failed to do so. In 7% (9/123) of cases, FC failed to formulate a diagnostic hypothesis owing to the sample inadequacy; 2 cases (2%) were not identified as lymphomas by FC (of them considered only “suggestive” also by cytomorphology); 1 case was not identified neither by FC, nor by cytomorphology. In cases having a histologic follow-up, levels of diagnostic sensitivity and specificity of the combination cytomorphology/FC were 97% and 94%, respectively.

Conclusions. FC is a fast and reliable methodology which easily detects disease-specific phenotypes and clonality in B-cell lymphomas. High-level multicolor FC has the advantage of being able to identify a small population of abnormal cells that may not be apparent on morphology alone. FC applied to FNA enhanced the diagnostic potential of cytologic diagnosis and subclassification of NHL, thus avoiding the need for invasive surgical biopsies in many cases.

Cytological and histological findings of florid papillary hyperplasia. A case report

S. Gorla, C. Di Bella
U.O. di Anatomia Patologica, Azienda Ospedaliera di Vimercate-Presidio di Desio

Introduction. Thyroid hyperplasia represents a benign condition which normally develops after a physiologic response of follicular epithelium to hormonal changes. Among these modifications a condition which may be the cause of diagnostic errors is represented by the florid papillary hyperplasia.

Material and methods. We report on a case of a 13 years-old girl with thyroid nodule (right lobe). No symptoms, clinical or strumal signs of hyperthroidism. Ecoguided fine needle (25 gauge) is made. In the specimen, immersed in fluid colloid, several fine papillary structures are present in aggregates sometimes tridimensional. The rich cellular component consists of follicular cells, sometimes similitudinous and sometimes with spinifollicular aspects and nuclear atypia, chromogranin and synaptophysin negative are found. The cytopathological finding is: tir3 (indeterminate). According to the guidelines, right euthyroïdectomy is made. Macroscopic finding: thyroid lobe with a well delimited nodule, with a diameter of 2,8 cm, hemorrhagic, centrally cystic and capsulated, placed in the lower pole. Hystologically, the nodule is made of follicular cells with variable diameter and surrounded by a continuous capsule. Into the lumen of the central cavity grow papillary structures. They are however lined by small, round cells that closely resemble normal follicular cells. The surrounding parenchyma also shows features of nodular hyperplasia, with hemorrhagic areas. These last probably caused by the passage of the needle. A further confirmation of the benignity of the proliferation, one should remind to test these lesions with CD15, HBME-1 and galectin-3. These markers would in fact result positive in malignant epithelial lesions and negative in hyperplastic lesions (benigne). Further antibodies used: CK19, calcitonin, CK34beta E12, cromogranine, sinapto, thyroglobulin.

Conclusions. Florid papillary hyperplasia, together with other cytological aspects as nuclear grooves and pseudoinclusions, clearing of nuclear chromatin, hypercromasia, nuclear pleomorphism with prominent nucleoli, eventually associated with stromal changes (psammoma bodies, dystrophic calcifications, myxoid degeneration, hemorrhage, can be the cause of important diagnostic mistakes. To a certain degree of experience it could be useful to associate the use of immunohistochemical markers which can be of great help in order to avoid wrong diagnosis, especially when particular nuclear aspects associated to the presence of papillary structures are present.

Acinic cell carcinoma of parotid gland with lymphoid stroma: a case presentation

S. Gorla, C. Di Bella
U.O. di Anatomia Patologica, Azienda Ospedaliera di Vimercate, Presidio di Desio

Introduction. Acinic cell carcinoma (Acc) is a rare low grade neoplasm of salivary gland and some extra salivary sites. It is the second most common salivary gland tumor in children next to mucoepidermoid neoplasm. A lymphoid stroma is a relatively frequent finding in salivary gland. In our case, the presence of prominent infiltrated lymphoid stroma led to difficulty in differentiating this tumor from other salivary gland neoplasm.
Method and results. We report a case of Acc of parotid gland associated with lymphoid stroma in a 58 year-old woman. An ultrasound guided fine needle biopsy (25 gauge) of the parotid nodule was performed. The specimen was represented by large aggregates of cuboidal epithelial cells organized in simil-acinar structures, sometimes loose, with basal nucleus and fine chromatinic pattern, evident nucleolus, and a background of diffuse heavy infiltrate of mature lymphocytes and few histiocytes. A total parotidectomy is made. At a macroscopic evaluation an apparently capsulated grey nodule of about 2,5 cm diameter is described. At the histologic evaluation the presence of acinic cell carcinoma with heavy limphoid stroma, papillary and clear cells patterns is confirmed. The acinic cell carcinoma can show several histological patterns (acinic cells, papillary microcystic, microfollicular, solid type, tubular-acinic, of intercalary ducts) combined with several architectural and cytoglycolic aspects (basophilic cells, osisiphilic cells, clear cells). The biological behaviour of Acc is unforeseeable. The presence of a prevalent solid component is strictly asociated to a very bad prognosis. Sometimes, in the acinic cell, a prominent infiltrated lymphoid stroma is associated, which in itself, has no prognostic significance; instead, as in our case, it would be a matter of neoplasms well circumscribed, arranged in a follicular pattern with low proliferative activity (Ki67: lower than 10%); the neoplastic component is totally plonged by limphoyd tissues within which were numerous well formed germinal centers, surrounded by a thin fibrous capsule.

Conclusions. Acc is an uncommonly encountered malignant neoplasms of the major salivary gland. Although rare (between 1% to 3% of all salivary gland), it’s very important to recognize Acc, because an accurate cytologic diagnosis lead to a proper surgical treatment reducing the rate of loco-regional metastasis.

Establishment of a pilot screening program for breast cancer in Beith Jala Government Hospital, Bethlehem

S. Guzzetti, L. Giordano*, A.M. Delpiano, P. Riella, L. Viberti
Department of Surgical Pathology, Valdese Hospital, ASL TO1, Turin, Italy; *CPO Piedmont, San Giovanni Battista Hospital, Turin, Italy

Introduction. A pilot screening program for breast cancer at Beith Jala Government Hospital (BJGH), Bethlehem started from January 2009, with the cooperation of the Palestinian Ministry of Health (MOH), the Italian Cooperation and the Italian NGO “Pathologists beyond Borders”. This project is part of a nationwide program sponsored by Italian Ministry of Foreign Affairs aimed to improve sanitary level in Palestine.

Methods. In BJGH was built a new Breast Screening Unit with a mammography set and an ultrasound machine. The Palestinian MOH launched an health educational campaign in Bethlehem area to raise awareness about the importance of early detection of breast cancer and educate health staff on clinical breast exam as first level of screening. Women with suspected breast lesions were referred to BJGH. When a lesion is detected, further diagnostic procedures and therapeutic measures are offered by BJGH Departments, namely fine needle aspiration (FNA) for cytology, surgery and oncological treatments. A database that collects all information about patients along all the diagnostic and therapeutic pathways is under way and will be made available to all units involved and to National Cancer Registry.

Results. During the first six months of screening 768 mammographies and/or ultrasounds were performed, 35 of which were considered suspected or positive (4.6%) and FNA was suggested. Eight mammographies (1.0%) were unsatisfactory for technical reasons. Out of those 35, FNA was actually performed for 23 women; six of which were positive (0.8%), 2 were diagnosed as benign but of uncertain malignant potential (0.3%), 9 were negative and 6 were unsatisfactory. In 15 out of 23 where it was possible to establish a correlation between the results of radiology and cytology, disregarding cases were radiological and/or cytological exams were unsatisfactory. There was a 93.3% concordance of cases (14 out of 15).

Conclusions. We consider this project as the first screening program in Palestine based on international guidelines. Despite its opportunistic model, the turn up of women for screening was surprisingly high. The equipment and the staff available at BJGH radiology and pathology departments allow a satisfactory level of diagnosis. Nevertheless, specialized training courses have been organized to enhance proficiency of the clinical and technical staff in early detection, accurate diagnosis and effective treatment of breast cancer.

Incidence of positive Pap smears between native and foreign population: the casistic of a public hospital in Turin with an high immigrants population rate

S. Guzzetti, L. Giordano*, A.M. Delpiano, P. Riella, L. Viberti
Department of Surgical Pathology, Valdese Hospital, ASL TO1, Turin, Italy; *CPO Piedmont, San Giovanni Battista Hospital, Turin, Italy

Introduction. Despite the City of Turin has a regular program of active screening for cervical cancer, since 1992 a considerable amount of women in and out the targeted age of screening (25-64 yrs) turns spontaneously to the local Health Centres for Pap smears. The Evangelico Valdese Hospital of Turin is not involved in the organised cervical screening program. It is situated in a district with a high percentage of foreign residents. According to the data provided by the Municipality of Turin, updated to 2008, the female reference population for our hospital is 217,355 (52.6% of the whole population), 22,599 of which are foreigners (10.4%). Aim of this study is to compare the incidence of positive Pap smears between Italian and foreigner’s women in order to check if there are significant differences.

Methods. We evaluated 17,883 consecutive Pap smears performed at our Hospital between 2005 and 2008, 3,239 of which (18.1%) belonging to not native Italian women. Using the database of the Hospital, we were able to separate women born in Italy from all other women. Pap smears were classified as “positive” according to the diagnostic definitions of Bethesda System 2001, thus excluding reactive cellular changes. The distribution of ages was similar for both groups, being 38.5 the median age of foreigner women (range 18-74) and 39.6 for Italian women (range 18-87).

Results. Basing on the place of birth, 14,644 (81.9%) Pap smears have been performed in native Italian women and 3,239 (18.1%) on foreigner women. Overall positive cases
Table I. Distribution of Pap smears diagnosis by women’s origin and grade of lesions.

<table>
<thead>
<tr>
<th></th>
<th>Pap smears in Italians</th>
<th>Rate</th>
<th>Pap smears in foreigners</th>
<th>Rate</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (unremarkable of inflammatory/reactive lesions)</td>
<td>13891</td>
<td>94.9%</td>
<td>3073</td>
<td>94.9%</td>
<td>16964</td>
</tr>
<tr>
<td>Low-grade lesions (ASCUS, ACUS, AGC, LSIL)</td>
<td>651</td>
<td>4.4%</td>
<td>130</td>
<td>4.0%</td>
<td>781</td>
</tr>
<tr>
<td>High-grade lesions (ASCH, HSIL, carcinoma)</td>
<td>102</td>
<td>0.7%</td>
<td>36</td>
<td>1.1%</td>
<td>138</td>
</tr>
<tr>
<td>TOTAL</td>
<td>14644</td>
<td>100.0%</td>
<td>3239</td>
<td>100.0%</td>
<td>17883</td>
</tr>
</tbody>
</table>

were 919 (5.1%), 166 of which (0.9%) performed in foreigner women. The incidence of positive cases in relation to the respective populations was 5.1% in both groups: 753 out of 14,644 for Italian women and 166 out of 3,239 for foreigner women. The distribution of diagnostic categories for the two groups is shown in Table I.

Conclusions. The incidence of positive Pap smears is surprisingly similar between the two groups, even for the distribution of diagnosis, with only a small increase of high-grade lesions among foreigner women. Obviously many social and behavioural factors could have contributed to these results, together with the particular characteristic of the two examined populations. Both native Italian or not native Italian women are probably well informed and sensitized about proper health care and act as an opportunistic screening group which spontaneously accesses to Health Centres for primary prevention.

Diagnostic value of microhistology in cytology samples from endometrial brushing

S.C. Anatomia Patologica, *S.C. Ginecologia e Ostetricia, Osp. Santa Croce ASLTO5, Moncalieri, Torino, Italy

Introduction. The efficacy of direct endometrial sampling in the collection of adequate and representative material is evaluated.

Methods. From 01-01-2008 to 31-05-2009 148 women (age 29-82, mean 56, 85 postmenopausal), underwent endometrial brushing with Endoflower® in the Department of Gynecology. All samplings were performed in an outpatient setting. 104 patients had abnormal uterine bleeding (of them 53 postmenopausal), 17 had asymptomatic endometrial thickening (> 4 mm), 5 had atypical endometrial cells on pap-smear, 8 patients needed preoperative controls for uterine prolapse, 7 were treated with tamoxifen and 7 had other problems. The collected samples were fixed in a solution containing alcohol, water, EDTA and KCO3 and then centrifuged. The supernatant was filtered and the pellet embedded in paraffin.

Results. All patients defined the technique rather painless. 3 cases suffered of shock. In 20 cases (13%) the sampling procedure was difficult due to cervical stenosis. A sample large enough to prepare a cell-block was obtained in all cases. In 21 cases (14%) the sample was non diagnostic. The cases were categorized as non-pathologic (negative) and pathologic (atypical and carcinoma). The correlation between cito-histology on samples obtained with brushing and histology on biopsy or surgical specimen was possible in 33 cases (23%), with a diagnostic concordance of 91%. The rate of inadequate biopsies was 27% (4/15). 11/13 malignant neoplasias (2 carcinosarcomas, 11 endometrioid adenocarcinomas) were correctly diagnosed in samples collected with endoflower. 1 adenocarcinoma and 1 carcinosarcoma were underrated as negatives. In the surgical specimen the adenocarcinoma was a small lesion on the tip of a polyp, while the carcinosarcoma showed a predominant intramural pattern of growth. A normal endometrium was interpreted on brushing as atypical hyperplasia. Sensitivity was 84%, specificity 95%, positive predictive value 92%, and negative predictive value 90%.

Conclusions. Endometrial direct sampling with Endoflower® device in an outpatient setting is well tolerated by the patient and well accepted by the gynaecologist. This procedure allows cell-blocks preparation. The endometrial cyto-histology is less expensive and invasive than other procedures and therefore it could be used in association with transvaginal sonography, even where liquid-based cytology is not in use.

Critical revision of inadequate thyroid fine needle aspiration (FNA) cytology in a single center

S.C. Anatomia Patologica, *S.C. Ginecologia e Ostetricia, Osp. Santa Croce ASLTO5, Moncalieri, Torino, Italy

Introduction. The percentage of inadequate samples in thyroid FNA ranges from 10 to 30% according to the literature. The management of these cases is still controversial. In this study the clinical and cytological features and the management of inadequate FNA collected in a single center are presented.

Methods. In the period January-1-2000, August-15-2009 (9,7 years) 1820 thyroid FNA were performed with ultrasound guidance at a Surgery Department of ASL TO 5 Piemonte Italy (population 280,000). The aspiration was performed, in an outpatient setting, by two alternate pathologists. They prepared the slides and assessed immediately the adequacy of the sample and afterwards they made the final cytologic diagnosis.

Results. A total of 90 patients (5%) had inadequate samples. Their mean age was 58 (range 21-83), the mean diameter of the nodule was cm 1.5 (range 0.7-4 cm). 60 of them underwent only one FNA and 30 had two FNA in the same session. No slides were rejected because of preparation artifacts. The causes of inadequacy were: excess of blood, that hampered further aspiration, (54 cases), few cells, less than 5 clusters of at least 10 follicular cells, (27 cases), missed sampling of the lesion with absence of epithelial cells (9 cases). 40/90 patients (44%) underwent a second FNA after 1 month. Five of them had again inadequate sample; 32 had a diagnosis of benign lesion (goiter), 3 had a diagnosis of follicular neoplasm and underwent surgery for histologic diagnosis (3 follicular adenomas). 10/90 patients (11%) directly underwent surgery that was already scheduled because of
a clinical diagnosis of troublesome bilateral multinodular goiter. 30/90 patients (33%) with benign clinical diagnosis and negative for familial thyroid disease had only ultrasound follow up. 10/90 patients (11%) were lost to the follow up.

**Conclusion.** The low percentage of non diagnostic material and the absence of preparation artifacts are likely due to the standardized method and to the presence of experienced pathologists in every step. In our experience the patients with inadequate FNA had benign lesions. This data confirms, as it is said in the literature, that in these cases a conservative approach with ultrasound follow up, is a suitable behaviour.

**Quality improvement in diagnostic hematopathology: an integration of human resources and of advancing technologies**


* Dept. of Pathology, Regina Elena National Cancer Institute, Rome, Italy; ** Hematology, Regina Elena National Cancer Institute, Rome, Italy; “ Clinical Pathology, Regina Elena National Cancer Institute, Rome, Italy

**Introduction.** Specific diagnostic fields in pathology subjected to frequent errors are lymphoproliferative lesions, pigmented skin lesions, breast biopsies and prostatic needle biopsies. In the last 8 years at the Regina Elena National Cancer Institute in Rome a major increase in diagnostic hematopathological workup was associated to the growing clinical hematological activity. The Certification ISO9001 has been recently achieved in the Pathology Dept. As part of the certification process, we started an analytical process in order to improve quality in hematopathology.

**Methods.** An immunohistochemical extended antibody panel is in our routine practice, providing immunophenotypic characterization of the different lymphoma entities. In Clinical Pathology, extensive characterization of lymphoid populations was obtained by flowcytometry analysis; moreover, Fluorescent In Situ Hybridization (FISH) cytogenetic analysis of bone marrow and in the Pathology Department, of formalin-fixed, paraffin embedded tissue was established for the major chromosomal aberrations in lymphoma. Ultimately, clonality studies in lymphoma diagnosis for both B- and T-cell proliferation have been established by capillary electrophoresis analysis of PCR products by using the BIOMED-2 protocol, and BCL2 rearrangement studies for Minimal Residual Disease (MRD) have been settled.

**Results.** The efforts in the immunophenotypic and genetic characterization of lymphoma cases on one side produced more accurate diagnoses. Selected diagnoses has been so far either spontaneously verified by the pathologists themselves or on clinician request or on patient demand by referring difficult or spontaneous verification by the pathologists themselves or on clinician request or on patient demand by referring difficult or controversial cases to major reference centers. However, old and new possibilities of error exist. Mechanisms to improve quality in diagnosis should be applied both to preanalytical and to analytical phases. Among relevant factors in the analytical phase, a second intrainstitutional review of the cases before sign-out of hematopathological diagnoses (primary diagnosis) has been recently established as a current procedure.

**Discussion.** Diagnoses in lymphoproliferative diseases have achieved a considerable laboratory complexity. For each new technology applied, new quality control procedures have to be applied. Weekly Multidisciplinary Disease Management Team (DMT) meetings provide discussion and agreement to every decision concerning hematological patients.

**Adrenal cortical carcinoma: from cytologic diagnosis to histological confirmation and prognosis evaluation**

J. Mazibrada, L. Daniele, G. Isolato, S. Barbero, D. Pacchioni, G. Bussolati

Department of Biomedical Sciences and Human Oncology, University of Turin, Italy

**Introduction.** Adrenocortical carcinoma is a rare neoplasm with a poor prognosis, with 16 to 38% of patients surviving for more than 5 years after diagnosis. It was recently reported that adjuvant mitotane may prolong survival in patients with resected adrenocortical carcinoma, increasing the need of an accurate diagnosis at an early stage. Ultrasound (US) guided FNAC (Fine Needle Aspiration Cytology) is gradually more being accepted as a means of pre-operatoratory diagnosis of adrenal cortical carcinomas.

**Methods.** We present a case of adrenal cortical carcinoma in a 39-year-old male patient presenting left flank pain and emaciation. The diagnosis was established by US guided FNAC on the basis of cytomorphologic and immunocytochemical characteristics that, after surgical treatment, were compared with the histological findings. In particular, the Weiss system criteria were evaluated on the surgical specimen to predict its clinical behaviour.

**Results.** The cytomorphological features included the presence of cells in sheets with a striking endocrine vascular pattern as well as the presence of single cells in a dispersed pattern. Immunocytochemically, the cells were positive for vimentin and synaptophysin and negative for chromogranin and cytokeratin. The proliferation index was of 30%. Some features such as the presence of necrosis and mitoses were compatible with the malignancy of the lesion, and a final cytological diagnosis of adrenocortical carcinoma was posed. The patient underwent surgical excision of the lesion; standard and advanced (macrosections, macrophotographs) analysis performed on the surgical specimen gave further insight in aggressiveness of the neoplasm. In particular, several parameters of bad prognosis such as high nuclear grade, atypical mitoses, necrosis, capsular and vascular invasion were present.

**Conclusions.** This case demonstrates the usefulness of FNA on US guidance biopsy in adrenal masses suspected for adrenal cortical carcinoma. Cytomorphologic diagnosis is based on a set of significant cyto logical features that have both diagnostic and prognostic significance.

**Malignant pseudothyroiditis induced by metastatic lung adenocarcinoma. Report of a case and review of the literature**


Division of Anatomic Pathology and * Div. of Nuclear Medicine, Catholic University, ** Div. of Endocrinology Santo Spirito Hospital, Rome, Italy

**Introduction.** Metastases to the thyroid gland are uncommon findings in clinical practice, although a frequency ranging...
1.2 to 24% of thyroid cancers in autopic and surgical series is reported. The commonest clinical picture of a metastatic spread to the gland is a rapidly enlarging nodule, with little or absent pain. A different setting is the endolymphatic spread of neoplastic cells within the thyroid parenchyma. This clinical picture resembling a subacute thyroiditis with satellite nodal enlargement was defined “malignant pseudothyroiditis” by Rosen and coll., in 1978. A case of malignant pseudothyroiditis is described and the literature is reviewed.

**Case description.** A 35 y.o. female patient presenting cervical bilateral nodal enlargement and pain in the posterior region, was admitted to the “A. Gemelli” University Hospital. She underwent a thyroid FNAB and the samples were processed with conventional and liquid based cytopathy. Immunocytochemical stainings showed a positivity for TTF-1, and CAM 5,2, and negativity for Thyroglobulin in the neoplastic cell cytoplasm. A diagnosis of malignant pseudothyroiditis induced by a lower right lung adenocarcinoma was made.

**Conclusions.** The routinely use of FNAB may reveal the metastatic nature of a thyroid lesion suspected of being a thyroiditis. Malignant pseudothyroiditis should be considered in presence of a frank neoplastic picture and in the absence of a thyroiditis.

**References**

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**Diagnosis of differentiated urothelial carcinoma: cytology + uCyt+TM.**

**Revision of cases of dipartimento anatomia patologica, Policlinico Universitario Bari**

A. Napoli, G. Napoli, L. Tantimonaco, R. Ricco

**Dipartimento Anatomia Patologica, Policlinico Universitario Bari**

**Introduction.** Urothelial bladder carcinoma is frequent in the male patients, with an age of incidence between 50 and 80 years. The main symptoms are hematuria and dysuria; the diagnosis is based on urinary cytology, pelvic ultrasound, cystoscopy and biopsy.

**Methods.** Urinary cytology has low sensitivity (50-70%) in well-differentiated forms and in an attempt to improve the diagnostic performance, we have associated conventional cytology with immunoassay uCyt+TM, direct immunofluorescence technique that uses marked antibodies against tumor associated markers. From March 2007 to August 2009 we have prepared in our laboratory, urine samples of 2700 patients (3 samples from each one, of 3 consecutive days). From the 3 urine samples were prepared 1 specimen colored with Papainou and 1 specimen treated with the immunostain uCyt+TM; observing both we made final diagnoses.

**Results.** 36 cases with a doubt diagnosis, which included: 14 cases with positive cytoscopy and histological diagnosis of “low-grade carcinoma”; 5 cases with negative cytoscopy and histological diagnosis of “aspecific cystitis”; in 17 cases the histological examination was not performed. 20 cases with cytological diagnosis of suspect, which included: 7 cases with positive cytoscopy and histological diagnosis of “low-grade carcinoma”; 6 cases with negative cytoscopy and histological diagnosis of inflammation; in 7 cases the histological examination was not performed. 53 cases with a positive diagnosis, which included: 16 cases with positive cystoscopy and histological diagnosis of “low-grade urothelial carcinoma of the bladder”; 5 cases with negative cystoscopy, positive ureteroscopy and histological diagnosis of “low-grade urothelial carcinoma of the pelvis”; 2 cases with negative cystoscopy and histological diagnosis of “prostatic adenocarcinoma”; 3 cases with negative cystoscopy for 2 years and then positive cystoscopy with histological diagnosis of “low-grade urothelial carcinoma”; in 25 cases histological examination was not performed.

**Conclusions.** The combination of conventional cytology + uCyt+TM increases the sensitivity in the diagnosis of urothelial carcinoma; it identifies patients with increased risk of relapse, it may replace cystoscopy in the follow-up of low-grade carcinoma; it is highly sensitive to detect urothelial cancers, of all grades and stages and it is capable of revealing cytological changes that are precursive of urothelial carcinomas, long before cystoscopy.

**References**

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**A rare case of retro-peritoneal ovarian granulosa cell-tumor (GCT) recurrence**

V. Nirchio*, N. Muscatiello, M. Di Maso, S. Principe, F. Diterlizzi, G. Verderosa, C. Panella, E. Ierardi

*U.O.S Cytopathology, departments of Pathology, Ospedali Riuniti, Foggia, Italy; Gastroenterology Unity Univ., Ospedali Riuniti, Foggia, Italy

**Introduction.** The granulosa cell tumors (GCTs) are rare neoplasm which originate from ovarian stroma. After surgery recurrence is very rare.

**Case report.** A 52 years old woman was admitted for sudden abdominal pain located in the mesogastrium with left and back irradiation.

It showed the characters of recurrence and was predominately nocturnal. Laboratory emergency tests showed an increase of ESV and RCP and successively neoplastic markers were negative. The patient history was silent until 2000 the patient underwent hysterectomy and bilateral ovariectomy. Histology revealed a picture of GCT.

**Discussion.** US scan showed pariaortic nodular clustered lesions expanding from the inferior margin of pancreatic head to the right renal hilus. The lesion dislocated towards the bottom right adrenal and kidney repositioning the pancreas and duodenum ventrally and cranially; it surrounded the cava inferior vein until imprinting renal vein. Overall TC picture was suggestive of retroperitoneal sarcoma. The consultant surgeon didn’t indicate the surgical removal of the lesion in consideration of the site of the lesion, the mass tendency to the infiltration of surrounding organs and vascular structure. Thus, the necessity of a histological diagnosis was absolutely necessary, for the oncologist, for the identification of chemotherapy. For this reason was performed EUS-FNA.

Cytology of aspirate was suggestive of a mesenchimal tumor with a low grade of malignancy.
A laparoscopic exploration was performed and a complete excision was of the neoplasm was possible. The final histological diagnosis was of retroperitoneal metastasis of GCT, with no local lymphonode involvement.

**Conclusion.** We emphasize the usefulness of EUS-FNA for either the diagnosis and a the correct anatomic definition of the lesion with the exclusion of the presence of neplastic infiltration of adjacent organs as well as the absence of vascular infiltration. Besides it is very important to have a correct indication for the most appropriate treatment that implies prognostic benefits for patients.

### The determination of P16 in thyroid lesions

V. Nirchio, M. Di Maso*, S. Prencipe*, F. Nirchio**, M. Zingrillo***

SSD Citopatologia Diagnostica, Dipart. di patologia clinica Azienda Universitaria-Ospedaliera, O.O.RR Foggia; *Medici Specializzandi, Azienda Universitaria-Ospedaliera, O.O.RR Foggia; **Senior Physicist Researcher, Ast.centro di Geodesia; ***Medico endocrinologo, libero professionista, Laboratorio Analisi “Napoletano” Foggia

**Introduction.** The determination of p16 protein in cancer of the uterine cervix has been widely documented in the literature. This is an inside control mechanism of each cell that switch by phase of quiescence to being replicated (mitotic’s phase). When immunohistochemical investigations shows a quantitative increase of p16 it indicates an extreme attempt to stop mitosis.

The aim of our study is to assess the percentage of p16 positivity in the group of patients with established diagnosis of papillary thyroid carcinoma, compared to a control group with benign lesions.

**Materials and methods.** In the period between January 2007 and May 2009, we examined n°490 needle aspirated thyroid. From 2008 we studied a second slide derived from the washing liquid of the syringe used to aspirate the needle that was set in a thin layer of the Thin Prep Cytic. All slides were stained with Papanicolaou’s coloring method. The cases studied were 21, divided into two groups: a group of 11 patients with cytological diagnosis of papillary thyroid carcinoma and a control group of 10 patients with benign thyroid lesions.

In the group of patients with cytological diagnosis of carcinoma, the diagnosis was also confirmed by histological exame performed after thyroidectomy.

All slides were职称ed and subjected to immunohistochemistry determination of p16.

**Results.** In the group of patients with papillary thyroid carcinoma the p16 positivity was 80%, with variable expression from 5% to 25% on the cell population examined. In the control group the determination of p16 was always negative.

**Conclusions.** The increase of p16 in papillary thyroid carcinoma always correlates with nodal metastasis. In benign lesions of the thyroid the determination of p16 was always negative.

**References:**


The concordance between pathologists in Pap test selected on the basis of clinical history and HPV-positive DNA test. Determination of p16 protein as a marker of viral integration and testing to increase the sensitivity of the Pap test in the liquid phase

V. Nirchio, F. Romano*, R. Clemente**, S. Fusilli***, D. Pedà*****, R. Antonetti*****

SSD di Citopatologia, Dipartimento di Patologia Clinica, Azienda Universitaria-Ospedaliera, O.O.RR Foggia; *Servizio di Anatomia Patologica IRCCS “CSS” San Giovanni Rotondo; **Servizio di Statistica IRCCS “CSS” San Giovanni Rotondo; ****Direzione Medica di Presidio, Azienda Universitaria-Ospedaliera O.O.RR di Foggia; *****Capo dipartimento di Patologia Clinica, Azienda Universitaria-Ospedaliera O.O.RR di Foggia

**Materials and methods.** Between August 2005 and May 2007 we studied 90 women with HPV infection. All patients have been performed to pap tests in a thin layer at in laboratory of Citopathology of O.O.RR of Foggia, a colposcopy and investigations of molecular biology for the typing dell’HPV. The cytological diagnosis, done by the Bethesda system in 2001, was compared by three different citopatologi experts. Patients with established diagnosis of HPV were compared with a control group with benign lesions. The increase of p16 in papillary thyroid carcinoma was performed as described by the company providing the kit p16 INK4a of Cintek (MTM). The determination of score nuclear of p16 was done with the parameters given in the study of Wentzensen N and C Bergeron, published in Cancer Cytopathology 2005.

**Results.** The patients studied were 86/90, the cases have been agreed 26/86 (18 negative, 6 LSIL, 2 HSIL): 30.2% respectively (20.9% negative, 7% LSIL, 2.3% HSIL). The statistic k (concordance index) value is very low, indeed k = 0.10 is found not to be statistically significant. By studying the cases related with the determination dell’HPV, is reduced to 61 cases, of which 6 (9.8%) is low risk, 55 (90.2%) high risk. The cases positive for HPV low-risk, reading by the three pathologists, were given the following interpretation diagnostics:

<table>
<thead>
<tr>
<th></th>
<th>Negativo</th>
<th>Ascus</th>
<th>LSIL</th>
<th>HSIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caso 1</td>
<td>2</td>
<td>1</td>
<td></td>
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<tr>
<td>Caso 2</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
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<td>Caso 3</td>
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<tr>
<td>Caso 5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Caso 6</td>
<td>1</td>
<td>2</td>
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<td></td>
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</tbody>
</table>

The 55 cases positive for HPV high risk are divides: 8 cases were considered to agree Negative, 5 cases agree LSIL and 42 discordant: 14.5%, 9.1%, 76.4% respectively of cases positive for HPV high-risk. The data are different by adding, in addition to morphological data, the determination of p16 protein,
in 85 patients analyzed, taking as the cut-off score 2 (below negative, positive above) we obtained: 78 (91.8%) agrees negative, 1 (1.2%) agrees positive, 6 (7%) disagree. Indeed in this case the statistic $k = 0.61$ with $p < 0.05$. This value of $k$ can be considered a good degree of correlation.  

**Conclusions.** The determination of p16 increases the diagnostic concordance between pathologists.

**References**  

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**FNAB of the salivary glands: role of the cytology and cyto-histological correlation**

V. Nirchio, M. Longo*, L. Magaldi*, M. Cassano*, P. Bufo**  
SSD Citopatologia Diagnostica, Dipartimento di Patologia Clinica, Azienda Universitaria-Ospedaliera OO.RR. Foggia; *S.C. di Otorinolaringoiatria Universitaria, Azienda Universitaria-Ospedaliera, OO.RR. Foggia; **S.C. di Anatomia Patologica Universitaria, Azienda Universitaria-Ospedaliera, OO.RR. Foggia

**Introduction.** The cancer of the salivary glands, in the western world, have got an incidence between 2.5-3% in 100,000 inhabitants a year. In most cases they are benign neoplasias, prevalently localized in the salivary parotid gland. The FNAB was spread by Martin and Ellis in 1930. In Europe, particularly in Scandinavia, it was spread faster than in the USA. It is a simple, cheap and rapid and rapid methodology.

**Methods.** In the period between February 2008 and May 2009, 39 patients, aged between 46 and 83, of whom 22 male patients went to the Laboratory of Cytopathology. All of them showed a symptomatology variable from one month to 1-2 years; they had undergone instrumental and laboratory tests in the Otolaryngologist University ward of the OO.RR. in Foggia and, waiting for the operation they have undergone aspirated needle by 23-25 G thin needle. The aspirated materials was smeared upon slides and fixed in 95% alcohol. The remaining material in the syringe liner was washed with cytolitic fixative liquid of Diatek Company and whirled in thin layer. All the compounds have been coloured with Papanicolaou method.

**Results.** The studied cases had the following distribution of diagnosis:

**Conclusions.** The comparison with the histological test, made at the Pathological Anatomy University Department of the OO.RR. in Foggia, pointed out an agreement of 88% for the benign lesions and of the malignant lesions.

The right diagnostic attribution within both the benign and the malignant neoplasias was of 81%. Such results, in our opinion, have been obtained thanks to the direct involvement of the pathologist with the clinician, on taking the sample. Moreover, the swiftness of the execution of the test has certainly favoured a profitable planning of the surgical work.

**References**  

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**The study of Cyclin A and Cyclin E as biomakers of the oncogenic activity of E6 and E7 of the HPV in the cervical intraepithelial neoplasia lesions**

A. Nocita, G. Pizzi, N. Papaleo, F. Musicò, S. Mazza, F. Tallarigo  
U.O. C. Anatomia Patologica, Ospedale S. Giovanni di Dio, ASP Crotone

**Introduction.** Integration of human papillomavirus (HPV) into the cell genome is considered to be an important event in the progression of the cervical neoplasia. The oncoproteins E6 and E7 are responsible of such event, as they are considered are potent cooperating oncogenes, since both the oncoprotein interact with the regulators of the cellular cycle: pRB and p53 altering checkpoint G1/S G2/M. These mechanisms are considered events key are in promoting the phase S of the cellular cycle, but above all in the synthesis of viral, indispensable proteins for the Viral DNA amplification. It is famous as (E6 and E7) can interact with at least 20 different proteins, including cell cycle regulators, transcription factors, and other metabolic factors.

In this study the attention has been focused on the grip interaction that sanctions or E6 that E7 with the regulating elements of the cellular cycle, and in more precise way on the indirect tie of E7 to the complexes Cyclin E/CDK2 and Cyclin A/CDK2, with successive degradation of: p21 and p27 that at last it determines the overexpression of the two Cyclin E and Cyclin A.

Cyclin E and Cdk2 assets, indeed the transcription and the activation of the Cdk2/cyclina complex and are had during the G1 phase. Cyclin E levels are high during late G1 and early S-phase in normal cells. Cyclin A it is a protein that is already revealable in phase S and its expression increases during the progression of the cellular cycle in G2 phase. Therefore both are considered of biomarkers of proliferation.

**Aim.** The aim of our study have been those. To examine the pattern of expression of Cyclin A and Cyclin E in the cervical lesions of high degree. To evidence their clinical implication in correlation with the histologic features in the cases of infec-

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<table>
<thead>
<tr>
<th>Pleomorphic Adenoma</th>
<th>Warthin tumor</th>
<th>Inflammatory</th>
<th>Malignant salivary gland tumor</th>
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<td>9</td>
<td>8</td>
<td>3</td>
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<td>5</td>
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<td>Lymph nodes intraparenchimal</td>
<td>Emangioma cavernoso</td>
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<td>2</td>
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tion from HP V. to monitor the oncogenic activity of E6 and E7 of the HPV genotypes

Methods. Serial sections (4µm thick) were prepared from 60 formalin-fixed, paraffin-embedd cervical biopsies [CIN 3 (24), CIN2 (21) and CIN1/2 (11)] and controls (4) in women of age comprised between the 20-45 that anticipate an anomalous Pap-test (L-SIL; H-SIL). To All the patients was performed identification HPV typing (DNA-mRNA). While the qualitative-quantitative identification immunohistochemistry the markers cyclin A and cyclin E is expressed in %.

Results. The data illustrated in table emphasize the results obtained from the immunohistochemistry analysis of cyclin A and cyclin E with correlation with the infection of the HPV types high-risk. From the data emerges that the expression of the cyclin E has an inversely proportional course to the degrees of the cervical intraepithelial neoplasia. In cases CIN 1/2(10 on 11), in fact, the positive values are of 43.50%; in cases CIN 2 (13 on the 21) values they diminish to 36.14%, until to reach 31.45% in the cases of CIN3 (18 on 24). While the overexpression of the Cyclin A increases in proportional to the degrees of cervical intraepithelial neoplasia (CIN 1/2, CIN 2 and CIN3). It is observed, in fact, than the levels of expression they are of 47.41% in the cases of CIN3 (18 on 24) and stretch diminish until to 36.42% in the cases of CIN 2 (14 on 21) and to 30.39% in the cases of CIN 1/2 (10 on 11). Of the 56 cases subordinates to immunohistochemistry surveying for research purposes of the markers: cyclin E cyclin A, are obtained from the immunohistochemistry analysis of cyclin and stretch diminish until to 36.42% in the cases of CIN 2 (14 on 21) and to 30.39% in the cases of CIN 1/2 (10 on 11). Of the 56 cases subordinates to immunohistochemistry surveying for research purposes of the markers: cyclin E cyclin A, are emerged that in 38 cases there a real and strong correlation between the two marker, of which (18) CIN 3, (10) CIN 2 and (10) CIN1/2. On the 38 correlated cases the determination of the mRNA is carried out then viral by means of the methodical NASBA. The search of the mRNA identifies the presence of the oncoproteins E6 and E7 that play a role key in the process of the cervical cancer. It is observable that the activity oncogenic mainly is expressed in the cervical lesions of high degree (CIN 3) and that the HPV16 is sure the more frequent and aggressive stock, since is revealed in 15 cases of CIN 3 and 8 cases of CIN2. It follows, in frequency, found HPV 18 in 3 cases CIN 3, 2 cases CIN 2 and in 3 cases of CIN 1/2. HPV 33, 31 and 45 are found alone in 7 cases of CIN 1/2.

Conclusions. The data illustrated evidence that the expression cyclin E is turned out useful in the interpretation of the histologic features, above all in the cases CIN 1/2, in which it affords to follow or the progression of the cellular cycle by now altered or the several phases of the replication cycle and DNA amplification of the various high risk HPV subtypes. The immunoeexpression of cyclin A, observed above all in 1 cases CIN 3 and CIN 2, is determined by the oncogenic activity of the oncoproteins E7 and E6 of the high-risk HPV types. E7 interacts directly with the Cdk2 substrate and indirectly with p21 and p27 (Cdk1) which are degraded by the same oncoprotein. In conclusion, therefore it can be deduced that the pattern of expression of cyclin E and cyclina A, it is an important factor prognostic of cervical carcinoma in early stages, allowing the use of these two antibodies as of the biomarkers profits of the oncogenic activity of the oncoproteins E6 and E7.

References

The biomolecular diagnosis in the benign lesions of the cervix uterina HPV-correlated.
The role of Test HPV-DNA
A. Nocita, I. Putrino, R. Primerano, F. Vitale, F. Vittimberga, M. Mauro, F. Tallarigo
U.O.C. Anatomia Patologica Ospedale S. Giovanni di Dio, ASP Crotone

Introduction. The infection from human Papillomavirus (HPV) represents the most frequent disease to sexual transmission, with a prevalence of the 70-80% of sexually active the adult population. They are identified beyond 100 types of HPV, which at least 17 can cause infections of the genital feature. Between these genotypes potentially to high-risk oncogenic (HPV 16-18-33-31-45 and those to intermediate risk there are HPV 39-52-51-56-58-59) Moreover in the same patient can be introduced of the multiple infections due to the contemporary presence of various genotypes of HPVs. The techniques of molecular biology have put on hand of the diagnostic routine of the valid instruments for the corrected identification of various subtype the viral ones, to they time associated to different levels of risk oncogenic. The study in question has estimated the clinical usefulness of the HPV High Risk Typing TM RG Real-Time PCR. Such methodical one is a test in a position to finding and of potentially typing in real-Time regime the virus presence oncogenic pertaining to the great family of the 16-18-31-33-35-39-45-51-52-56-58-59 HPV response of the cancer of the cervix but also in the cervical lesion benign.

Methods. In biennium 2007 -2009 in the Service of Anatomy Pathologica and Cytologica 350 sample of cervical biopsies, pertaining to women of age comprised between the 20-50 are examined sexually active years and from induced cervical pathology from virus HPV. The samples of cervical biopsies are executed in colposcopical sitting; In 211 cases it is made diagnosis is of lesions of low degree (CIN1) that of lesion of squamous metaplasia. Successively all these patients have been subordinates to an ulcer cell sample in liquid phase in order to HPV DNA detection. A direct amplification of HPV DNA extracted is carried out by means of uses it of primer situated specific, respective for the codifying sequences the L1 protein. The use of the set of primers specific for the premature sequences affords

<table>
<thead>
<tr>
<th>Diagnostic parameters</th>
<th>Cyclin E</th>
<th>Cyclin A</th>
<th>Correlation-HPV</th>
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<tbody>
<tr>
<td>CIN3 n = 24</td>
<td>31.30% (18/24)</td>
<td>47.41% (21/24)</td>
<td>15 HPV 16; 3 HPV18</td>
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<tr>
<td>CIN 2 n = 21</td>
<td>36.14% (14/21)</td>
<td>36.42% (14/21)</td>
<td>8 HPV16; 2HPV 18</td>
</tr>
<tr>
<td>CIN1/2 n = 11</td>
<td>43.50% (10/11)</td>
<td>30.39% (10/11)</td>
<td>3 HPV 33; 3 HPV 18; 3</td>
</tr>
<tr>
<td>Controls n = 4</td>
<td>0%</td>
<td>0%</td>
<td>HPV45; 1 HPV 31</td>
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<td>Total n = 60</td>
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to discriminate the viruses pertaining to the group low and high/medium risk oncogenic.

Results. The obtained results are brought back in Table I.

Conclusions. Our results show that the Dysplasia of low degree (CIN 1) is caused in particular by double or the multi-ple infections, which they are genotypes to intermediate risk mostly, like HPV types 52-56-58. This HPV types (52-56-58) have got in the regions “ORF” homologous nucleotydiche sequences to types of HPV 16-18, this explain because these genotypes are found only. While the koilocytotic changes in cervical squamous epithelium, represent the first observable clinical manifestations of HPV infection, in fact caused pri-marily by HPV types 51-35-59-39.

From this study it can be deduced that the application of the HPV-DNA test also on the benign lesions reveals an effective method for a corrected diagnosis, and that at the same time it allows to more execute successively on these cases the controls adapted clinicians. Indeed a positive test to the HPV does not mean necessarily that a woman will develop to a cervical cancer but she supplies profits more information for the taken care of controls. On the contrary the outcome negative of HPV DNA test is able to exclude with good reliability the presence of lesions of high degree and therefore it offers to the patient which necessary psychological comfort in facing the following follow-up.

References


FNA cytology diagnosis of phyllodes tumour of the breast: ASLTO5 experience

J. Prestipino, F. Pietribiasi, C. Manini, D. Stramignoni

Servizio di Anatomia Patologica ASLTO5 Ospedale Santa Croce Moncalieri, Torino, Italia

Phyllodes tumour is an uncommon fibroepithelial tumour of the breast. The main diagnostic issue is to distinguish it from fibroadenoma. The differential diagnosis is particularly difficult in fine needle aspiration (FNA) cytology. The experience of Servizio di Anatomia Patologica ASLTO5 Regione Piemonte of FNA cytology of phyllodes tumour is reported.

From year 2000 to year 2009, 43 patients underwent surgical excision of a phyllodes tumour. A previous FNA cytology diagnosis was performed in 21 cases. Among them 2 were malignant and 1 “borderline”. The age of the patients ranged from 21 years to 69 years (mean 39.6), being the patients with malignant and borderline tumours older (mean 50 years). The size of the benign lesions ranged from cm 1.8 to cm 15 (mean cm 4.3); the 2 malignant tumours measured cm 5.6 and cm 13. The cytological diagnosis was phyllodes tumour in 11 cases (52%), in 2 cases the diagnosis was: benign cyst (in one of them an area of cystic degeneration was present) and in 8 cases (38%) the diagnosis was fibroadenoma. In 3 of the latter cases areas of fibroadenoma-like were detected in the surgical specimen. Malignancy was not diagnosed, although atypical cells were reported.

Our experience is in keeping with the data of the literature where the accuracy of FNA cytology diagnosis ranges from 27 to 97%. The main difficulty is the differential diagnosis with fibroadenoma.

We conclude that extensive FNA sampling including different areas of the lesion should improve the accuracy of FNA cytology diagnosis of phyllodes tumour. However mammography and ultrasound pattern and clinical presentation must be considered in conjunction with FNA cytology in order to achieve a reliable preoperative diagnosis.
A rare case of warthin-like papillary carcinoma of thyroid, oncocytoid tall cell variant. Case report and cyto-histological correlations

A. Rasi, P. Rossi, M. Ballotta, L. Borghi
Dipartimento di Patologia Clinica. S.O.C. Anatomia Patologica. Azienda ULSS 18-Rovigo

Introduction. Tall cell variant of papillary Hurthle cell carcinoma with lymphoplasmacytic stroma is an uncommon and less aggressive form of papillary carcinoma that is associated with a long-term favourable prognosis. This variant tends to affect older patients more often than the conventional form.

Methods and results. A 73-year-old woman, with a history of goiter, presented in October 2002 with thyroid enlargement and significant tracheal deviation. The ultrasound report revealed multiple isoechoic nodules with calcifications in the right lobe. FNAC yielded cellular smears consisting of a nodular goiter with presence of papillary fronds of tumour cells mixed with lymphoplasmacytic cells. There were also small, monolayered groups as well as scattered isolated tumour cells. The nuclei were usually eccentric, large, round to oval. Nuclear grooves were seen in papillary clusters but not in isolated cells. Nuclear inclusions were few. Same small lymphocytes were present in the background. Mitosis was not observed. The diagnosis was of suspicious lesion. In February 2004 the patient underwent total thyroidectomy. In the goiter there was a neoplastic white and firm 1.5 cm in diameter nodule. Histologically two distinct patterns were present: nodular goiter and papillary tumour with oncocytic features. The neoplasm was characterized by a papillary growth of oncocytic cells with classic nuclear features of papillary carcinoma with brisk lymphoplasmacytic infiltration in the papillary stalks. Same follicles were lined by tall cells with eosinophilic and less granular cytoplasm than Hurthle cells, with not very prominent nucleoli. Multinucleated giant cells were showed. IHC: the epithelial cells were negative for calcitonin, positive for chromogranin, synaptophysin, thyroglobulin, CK7 e CK20, Ki67 MIB-1: 3%. S100 positive dendritic stromal cells were present. A diagnosis of papillary Hurthle cell carcinoma with lymphoid stroma was made. (pT1). In April 2009 the patient was living and disease free.

Conclusions. Warthin-like tumours can be mistaken for benign lymphoepithelial lesions of the thyroid, Hurthle cell carcinoma and the tall cell variant, which is a very aggressive neoplasm. In our case the tumour showed less than 70% tall cells in a background of oncocytic papillary neoplasm with lymphoid stroma, so we prefer a diagnosis of tall cell Warthin-like papillary carcinoma, a distinct entity with favourable prognosis.

References

Breast metastasis to the thyroid diagnosed by fine-needle aspiration: a case report

A. Rasi, P. Rossi, M. Ballotta, L. Borghi
Dipartimento di Patologia Clinica. S.O.C. Anatomia Patologica. Azienda ULSS 18-Rovigo

Introduction. Metastases to the thyroid gland are rarely encountered in clinical practice and may mimic primary tumours. Kidney, lung, breast, esophagus and uterus are the most common primary sites.

Methods and results. A 61-yr-old female presented with rapidly enlarging firm mass in the right lobe of the thyroid gland. The thyroid lesion had a hypoechogenic pattern at the sonographic examination and did not take up the radioiodine. The hormonal workup showed no clinically relevant alteration. Fine-needle aspiration revealed a cellular smear, many loosely cohesive and individual scattered pleomorphic malignant cells, sometimes arranged in three-dimensional clusters, syncytial groupings and occasional acinar patterns, in absence of benign thyroid epithelial cells and colloid. The background was full of debris. The cells showed enlarged irregular nuclei with hypercromatism. Immunohistochemistry was negative for thyroglobulin and positive for CK7. A metastatic adenocarcinoma of unknown origin was diagnosed. The CT showed a 2 cm nodule on the right breast. The core biopsy confirmed the diagnosis of breast ductal cancer. The patient received chemotherapy. One year later she presented multiple brain and bone lesions and died of disease.

Conclusions. Secondary involvement of the thyroid gland from a remote primary malignancy is uncommon. Breast cancer is one of the most common tumour which metastasizes to the thyroid. Thyroid metastases usually occur when there are metastases elsewhere, sometimes many years after the diagnosis of the original primary tumour. Any thyroid nodule in a patient with a previous history of cancer needs to be evaluated. In our case, thyroid metastasis was the first sign of the breast primary cancer.

References

Comparison between conventional and liquid-based cytology for the diagnosis of thyroiditis on fine-needle aspiration biopsy

E.D. Rossi, L. Santoro, S. Moncelsi, G. Chiarello, G.F. Zannoni, G. Fadda
Division of Anatomic Pathology and Histology, Catholic University, “Agostino Gemelli” School of Medicine, Rome, Italy

Introduction. The efficacy of thyroid fine needle aspiration (FNA) in diagnosing Hashimoto’s Thyroiditis (HT) on liquid-based cytology (LBC) and Conventional smears (CS) is evaluated in two reference periods. In the triennium 1996-1998 the cytologic cases were processed only with CS, in 2004-2006 only with LBC. The diagnostic features of the hyperplastic nodules in HT and the possible pitfalls are discussed.

Materials and methods. In 1996-1998 150 cases diagnosed as HT at the “Agostino Gemelli” Hospital of Rome had CS only whereas 463 cases in 2004-2006 had only LBC. The slides for CS were fixed in ethanol and stained with Papanicolaou. For LBC the aspirated cells were submerged in the alcohol-based solution Cytolit (Hologic, Marlborough, USA) then processed with the T2000 processor (Hologic) and stained with Papanicolaou.

Results. Among the 150 cases of the first triennium 83 were cytologically HT while 67 were HON; in the second triennium 201 were HT and 262 were HON. For the first period a follow-up (including a second FNA or surgery) was done...
in 92 cases and in the second period in 97. In the first period among the 44 HON 2 (4.6%) resulted malignant whereas in the second period 5 out of 52 (9.6%) were malignant. When a diagnosis of HT was made, only 1 case in the second period (2.6%) resulted malignant.

Conclusions. LBC can be adopted as a valid alternative method in the difficult cytological diagnosis of HT and hyperplastic nodules in HT. It may be useful for the appropriate clinical management of the patients affected by nodular thyroiditis.

References

A rare case of peritoneal metastases of well differentiated papillary thyroid carcinoma. A case report

P. Rossi, A. Rasi, M., Ballotta, L. Borghi, E. Bianchini
Dipartimento di Patologia Clinica. S.O.C. Anatomia Patologica. Azienda ULSS 18-Rovigo

Introduction. Metastases from papillary thyroid carcinoma at unusual sites are typical of dedifferentiation and often arise several years from onset. We report a case of peritoneal metastases from well differentiated papillary thyroid carcinoma, which maintains the same degree of differentiation of the primary neoplasms.

Methods and results. In March 2008, a 66-year old man was admitted to our hospital for peritoneal effusion. Past medical history revealed a total thyroidectomy with right cervical lymphadenectomy for classic papillary carcinoma stage T3 N1b, five months ago, treated with I131 therapy. At ultrasound the liver was enlarged but biliary tract and other abdominal organs were normal. Cytological examination of peritoneal effusion showed small, compact, pseudopapillary clusters of small cells. Because of the absence of nuclear infolding, nuclear holes, and psammoma bodies and the presence of tall-columnar neoplastic cells, sometimes with clear cytoplasm, a peritoneal biopsy was performed in order to exclude a mesothelial neoplasia. On histological section the pattern consisted of classic papillary carcinoma with pseudoglandular aspects, tall columnar cells, focal clear cell areas and psammoma bodies. Neoplastic cells showed mild mitotic activity and a growth fraction (ki 67) of 15%. Immunoistochemistry was negative for mesothelial markers (calretinin, CK 5-6) but a strong positivity for CK 7, TTF-1 and thyroglobulin was demonstrated. These data supported a diagnosis of peritoneal metastases from papillary thyroid carcinoma. No recurrence was demonstrated at follow up.

Conclusions. Involvement of cervical lymph nodes is very common and it may be the first manifestation of the disease. Blood-borne metastases are less frequent but they also occur and the most common site is the lung. Metastases at unusual sites are typical of dedifferentiation while in our case the degree of differentiation was the same of the primary neoplasm.

References

Intralaboratory quality controls in a screening centre for the prevention of cervical tumors

T. Rubino, R. Bio, L. Campioli, B. Aguzzoni, S. Prandi
Centro di Citologia Cervico-vaginale, Reggio Emilia, Italia

This is an evaluation of the efficacy and applicability of Intralaboratory Quality Controls (IQC) in a medium sized Cervicovaginal Cytoscreening Centre. The Cytology Centre is a standardized laboratory of Reggio E. with a screening load of about 39000 Pap tests per year for diagnostic levels 1 and 2, with 4 full-time cytotechnologists, 2 supervisors. With the number of cytotechnologists at the limit of individual possibilities, those with an excellent cost/benefit ratio are analyzed in the choice of intralaboratory quality controls: the main target is the reproducibility and the accuracy. The first objective is to homogenize the number of slides read by each cytotechnologist, with a random distribution for the 1st diagnostic level. Thus, the evaluation of the comparison between the percentage of diagnostic categories from the number of unsatisfactory Pap tests, with the pathological ones, carried out at 3, 6, 9 and 12 months, assumes importance in the understanding of the laboratory’s activities, but provide a stimulus for improvement in bringing consistency to diagnostic standards. The attribution of level 2 to the cytotechnologist who diagnosed the lesion and proceeds with the follow-up, leads to a continuous comparison of the diagnostic choices made which are integrated with the cytohistological correlations in such a manner that the comparison is no longer an operation done afterwards. Peer review of the complicated cases leads to individual reflection. Review of the FN and FP is indispensible, while the review of the unsatisfactory Pap tests is indicated when percentage deviations are observed. Re-reading of 10% of the slides in order to find FN is futile; in our opinion, it is significant for improving the diagnosis of negative/phlogosis classes.

Conclusions. On the basis of our experience, here are some suggestions for the IQC: 1) cytohistologists meant only for reading, with = n° of slides; 2) positive cases studied throughout the follow-up; 3) statistical evaluation; 4) peer reading integrated with the cytohistological correlations; 5) periodic reviews of 10% of slides. Each measurement mentioned above has a cost and the choice to be made depends on the dimensions of the laboratory.

References

Synergy of cytological methods in assessing lymph node status of breast carcinoma patients: preoperative ultrasonography-guided fine needle aspiration and intraoperative sentinel lymph-node evaluation

F. Saro, S. Lanata, M.C. Aquilano, M.G. Sironi
S.O.C. Anatomia e Istologia Patologica, P.O. Sestri Levante

Introduction. Axillary lymph node status is a recognized prognostic factor that influences the therapeutic management of breast carcinoma patients.
Ultrasonography-guided fine needle aspiration (US-FNA) of suspicious axillary lymph nodes is a sensitive method to determine axillary lymph node involvement during preliminary staging of breast carcinoma. Associated, if negative or indeterminate, with cytological intraoperative sentinel lymph node examination (iSLN), allows to select those patients who can really benefit from conservative treatment.

Methods. 377 women with breast carcinoma were received to our hospital, from 2005 to 2009. US-FNA of ultrasonographically suspicious axillary lymph nodes was performed on 150 women; 96 had subsequent histological confirmation of the nodal status, while 22 were lost to follow-up and 30, with positive tests and advanced stage disease, were recommended chemotherapy treatment. 302 patients, including the 96 with negative or indeterminate US-FNA, underwent iSLN evaluation by scraping cytology of the two halves of every axillary SLN submitted. Sensibility and predictive values of both methods were calculated.

Results. The negative predictive value (NPV) of the US-FNA was 84%, the accuracy was of 94%, with a total of 32 avoided iSLN (78% of all complete axillary dissections), due to positive US-FNA.

iSLN sensibility was 98%, as compared with the histological definitive sections (55/56), while the NPV (92%) was influenced by the presence of micrometastasis, not visible in the cytological smear, after review of the preparations.

Conclusions. Our study confirms that US-FNA is a simple and reliable technique in nodal preoperative staging of breast carcinomas, allowing to adequately and efficiently select whose patients can really benefit from the iSLN technique, and furthermore validates the effectiveness of the cytological intraoperative evaluation of sentinel lymph nodes, with high sensibility and predictive values.

Diagnosis of lymphomas by eus-guided fine needle aspiration combined with flow cytometry analysis. A retrospective study


Flow Cytometry Unit, Molinette Hospital, Turin; * Laboratory of Pathology, Molinette Hospital, Turin; ** Department of Gastroepatology, University of Turin; *** Department of Biomedical Sciences and Human Oncology, University of Turin, Italy

Introduction. Endoscopic ultrasound combined with fine needle aspiration (EUS-FNA) is rapidly becoming the preferred diagnostic approach to sample masses in mediastinum, upper gastrointestinal tract, pancreas and liver. In the past, EUS-FNAB has prevalently been applied to the diagnosis and staging of solid cancer while its accuracy in the diagnosis of lymphoproliferative diseases (LD) is less defined. Flow cytometry (FC) has successfully overcome many of the limits of cytology in the diagnosis of LD and its application to EUS-FNAB samples as a tool to improve overall sensitivity and specificity in patients with suspected LD is promising.

Materials and methods. Multicolour FC was performed on 29 EUS-FNA specimens from patients with clinically suspected LD between July 2004 and January 2009. Sample was obtained by nodal (22 cases) and extranodal (7 cases) tissue. A definite histopathological diagnosis on surgical material was obtained in 4/9 patient affected by NHL.

Results. In 7/29 patients (25%) with clinically suspected LD, cytological studies eventually disclosed metastatic carcinoma. A diagnosis of mediastinal extramedullary emopoiesis was raised in one additional patient. Among the 21 remaining subjects, a definite diagnosis of lymphoma (8 B-cell NHL and 1 T-cell NHL) or reactive lymphoadenopathy (12 cases) could be obtained by cytology alone or aided by immunocitochemical studies on cell block in 12/21 (57%) of cases and by FC alone in 20/21 (95%) of cases. In 3/9 (33%) of cases the cytological and immunocytometry studies could also unequivocally lead to the specific histotype while a specific histotype could be defined by FC alone in 56% (5/9) of cases. FC could not identify the histotype in two diffuse large B-cell lymphomas (DLBCL) because cells displayed physical scattering features intermediate and in one follicular lymphoma (FL) that was CD10 negative. One additional case of suspected T-cell NHL could not be definitely diagnosed based on FC findings alone. Consequently, the sensitivity and negative predictive value of FC for the diagnosis of lymphoma was higher than that of cytology combined with immunocytometry (88% and 91% vs 64% and 75%, respectively).

Conclusions. FC applied to EUS-FNA is a feasible and highly accurate method for the diagnosis and subtyping of deep-seated lymphoma and can significantly improve the performance of cytomorphology in the diagnostic evaluation and treatment decision.

Prognostic markers in breast carcinoma on liquid based cytology

M.C. Truglia, L.R. Girardi, A. Giannini

U.O. Anatomia Patolologica Ospedale “Misericordia e Dolce”, Prato, Italy

Introduction. The aim of the study is to standardize the technique of immunocytocchemical (ICC) assessment of progra-
tic markers in breast carcinoma and to compare the results with immunohistochemistry on paraffin blocks.

Methods. On 46 cases of breast carcinoma diagnosed by fine needle aspiration cytology (FNAC) ICC assessment for Prognostic markers (ER, PGR, Ki67, Her2) was carried out. The results were compared to those obtained from immunohistochemical (IHC) evaluation of the prognostic markers in section of paraffin blocks obtained from the same breast tumor.

Results. Prognostic markers positivity rate was compared between the liquid based cytology and the tissue sections. In 2 cases the cellularity was insufficient to assess makers expression. One case was ER negative on histology and positive (40%) on cytology. We haven’t found differences in the expression of markers in ICC and IHC.

The concordance between cytology and histology was 94% but positivity rate were higher in cytotechnology than in histotechnology technique.

Conclusion. FNAC and ICC are simple and rapid techniques furthermore the antigenity loss due to fixation and processing methods is minimal, compared to histological set up. Therefore it seems to improve the sensitivity of the detection of ER positive cases, playing an important role both in the stratification of the risk and in the therapeutic decision making in patients with breast cancer.

3 days urine collections in one, an improved procedure

E. Valagussa, P. Libtretti, S. Castriciano

Cytology Department, Fleming Labs, Brescia, Italy; *Copan Italia S.p.A., Brescia, Italy

Background. Urine cytology is used for the diagnosis of tumors and cancer of the urinary tract system, including cancer of the bladder, urethra, ureters and kidneys. Urine collection is non-invasive, but has the disadvantages of daily collection for 3 days and must be processed soon after collection to assure high quality of cell preservation. Copan (Brescia, Italy) developed a urine preservation medium (UPM) that stabilizes urine contents for longer periods of time and allows 3 days collection in the same container. Our objective was to compare urinary cell preservation for cytology in UPM, 3 days urine collection in one container, to a urine collection per day for 3 days in single vials of ThinPrep medium (TP) (Hologic).

Methods. Urine samples were collected for 3 days from 50 patients; each day 30 ml of urine was added to a vial of TP and 20 ml of urine was added to a vial containing 30 ml UPM. The sediment obtained by centrifugation at 1500 rpm for 10 minutes was aliquoted to prepare both cyto-spins and cell-blocks (CB). The presence of tubular cells was assessed by standard Papanicolaou staining. CBs were employed to stain tubular cells for their antigenity loss due to fixation and processing methods is minimal, compared to histological set up. Therefore it seems to improve the sensitivity of the detection of ER positive cases, playing an important role both in the stratification of the risk and in the therapeutic decision making in patients with breast cancer.

The detection of tubular cells in urine cytology is associated with contrast-induced nephropathy

A. Zabatta, A. Iaccarino, R. Boschi, G. Troncone, A. Vetrani, C. Briguori, G. Condorelli

Depts of Biomorphological and Functional Sciences, *Cellular and Molecular Biology and Pathology, “Federico II” University of Naples

Aims. Percutaneous coronary investigation (PCI) may be complicated by contrast-induced nephropathy (CIN). We have previously shown that contrast agents directly induce renal tubular cell cytotoxic changes in animal models. The aim of this study was to evaluate whether similar changes also occur in patients undergoing PCI. Methods: In this subgroup study urine samples were collected by catheter from 22 patients before and after (6h and 24h) the administration of contrast medium. The sediment obtained by centrifugation at 1500 rpm for 10 minutes was aliquoted to prepare both cyto-spins and cell-blocks (CB). The presence of tubular cells was assessed by standard Papanicolaou staining. CBs were employed to stain tubular cells for their antigenity loss due to fixation and processing methods is minimal, compared to histological set up. Therefore it seems to improve the sensitivity of the detection of ER positive cases, playing an important role both in the stratification of the risk and in the therapeutic decision making in patients with breast cancer.

Results. Most of the specimens collected before PCI (64%) did not show tubular cells; conversely, tubular cells, also highlighted by Gal-3 positivity, occurred in most cases following PCI (48% after 6 hours; 71% after 24h). Tubular cells occurred in clusters and casts and showed clear or vacuolated cytoplasm, intracytoplasmic pigmented granules and nuclear changes. Reactivity for caspase-3, p53 and p21WAF1 was associated with this morphology.

Conclusion. The presence of apoptotic tubular cells may have important implications for the clinical management of patients undergoing a PCI. Their detection may reflect an undergoing kidney damage. Thus, urine cytology may play a role in monitoring the extent of tubular damage in CIN.

References