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An ultrastructural study of the histogenesis of haemangioblastoma

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Key words
Haemangioblastoma • Lipid droplets • Mesenchymal stem cells

Summary
Seven cases of cerebellar haemangioblastoma, not associated with von Hippel-Lindau disease (sporadic haemangioblastomas), were studied by light and electron transmission microscopy. Morphological features that might provide information about the histogenesis of the tumour were examined. The ultrastructural data indicate both the common ancestry of the different cytotypes that make up the tumour, and the mesenchymal origin of the elements present, which were also documented by their capacity to synthesise lipid droplets in the cytoplasm (a process of lipidization similar to that of pre-adipocytes).

Introduction
Haemangioblastoma is a slow-growing tumour of uncertain histogenesis composed of small-sized vessels and stromal cells; in the central nervous system, it arises with a greater incidence in cerebellar hemispheres. It can appear as either a sporadic form or may be associated with von Hippel-Lindau disease. It first appears as a solid nodule, but during its growth it undergoes numerous confluent excavations that become micro and macropseudocysts. By microscopic investigation, it is composed of well-structured capillaries and stromal cells. On the basis of the predominance of one of these two components and/or quantitative equivalence, three subtypes have been identified: 1) vascular form; 2) cellular form and 3) mixed or typical form. The vessels have a simplified architecture, variable diameter and may show a cavernous appearance. They are very fragile and easily permeated; this structural condition is one of the factors that play a role in the formation of haemorrhagic foci and transudation often found in these tumours.

The stromal cells are phenotypically diverse: egg-shaped, spindle-shaped, roundish, with a transparent cytoplasm and different levels of rarefaction and frequent lipidization; although irregular, nuclei do not show atypical features or morphological signs of proliferation.

It is well known on a morphological-descriptive level that this tumour is somewhat puzzling on a histogenetic level as the nature of the stromal cells is still unknown. This uncertainty has prompted different hypotheses regarding their origin, but the strongest theory sustains that the elements that make up the above-mentioned vessels (endothelia and pericytes) and stromal cells derive from a single cell type. Other documented histogenetic hypotheses have also been forwarded.

From a review of the literature, different theories emerge: the tumour has been considered to have a neuroectodermal nature, endothelial origin, arachnoid genesis or a fibro-histiocyte type, or more simply of variable or heterogeneous origin.

Recently, a protein also present in progenitor cells of the embryonic haemangioblastoma was isolated from stromal cells in this tumour, and was considered to be a factor favouring its vasculo-embryonic nature. However, this finding does not eliminate all uncertainties surrounding this neoplasm.

To shed light on the histogenesis of haemangioblastomas, an ultrastructural analysis of haemangioblastoma was carried out.

Materials and methods
Seven cases of cerebellar haemangioblastoma, not associated with von Hippel-Lindau disease (sporadic haemangioblastoma) were studied. The patients, 2 woman
and 5 men, ranged in age from 36 to 74 years (Table I). Serial cuts were made from each sample and were investigated by either light or electron microscopy. For the former, samples were fixed in 10% formalin, diluted with a 0.1M phosphate buffer, pH 7.2, and after sufficient dehydration were embedded in histowax; 5-micron sections were cut from these blocks and treated as follows:

a. histopathology: haematoxylin-eosin, H-v.Gieson, silver staining according to Gomori, Azan-Mallory trichrome method;

b. immunohistochemical methods: the following antibodies were used: vimentin (Dako, 1:200), desmin (Dako, 1:100), CD34 (Dako, 1:50), GFAP (Dako, 1:200), S100 (Dako, 1:200) E.M.A (Dako, 1:200), N.S.E. (Dako, 1:100), cytokeratin and keratin (56 kDa and 64k Da, Dako, 1:100).

The fragments for electron microscopy were fixed in Karnovsky’s solution, post-fixed in 1% osmium tetroxide, dehydrated in alcohol and embedded in Epon 812 resin. From these blocks, semi-fine sections were cut and stained with toluidine blue, after which ultra-fine sections were cut and contrasted with lead citrate and uranyl acetate.

### Results

#### Light microscopy

The cases under examination generally presented a microcystic architecture due to the presence of small wall-less cavities containing acidophilic amorphous material. The areas lying between the cavities appeared compact or slightly cribrose since they were made up of small-sized vessels surrounded by varying densities of stromal cells. The vascular component was composed of capillaries with different sized lumen, which were all well structured and each was lined with endothelia resting on a thin basal membrane surrounded by pericyte elements (Fig. 1). Stromal cells filled the interstitial space and surrounded the vessels; they were in mutual contact, medium-sized and polygonal or spindle-shaped; each had a large, clear cytoplasm, often containing clusters of lipid droplets in varying quantities, and a large vesicular nucleus at the centre (Fig. 2). These structures were divided by a loosely-knit network of collagen fibrils that acted as a

<table>
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<tr>
<th>Gender</th>
<th>Age</th>
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support and separated them in lobules. Haemorrhagic foci, interstitial oedema and severe hydropic swelling and cytolysis of cells were very frequently visible. In all cases, immunohistochemistry showed positivity for CD34 of endothelia and vimentin positivity of stromal cells. The markers for the remaining antigens tested (S100, N.S.E, GFAP, E.M.A., desmin, keratin) were negative.

**Ultrastructural findings**

*a. Endothelia*

The endothelia lining the capillaries appeared large and bloated and invading the lumen; their cytoplasms appeared diaphanous, had scanty organelles and often contained lipid droplets. They were single-layered and rested on a basal membrane of varying thickness (Fig. 3).

*b. Pericytes*

The pericytes were pseudostratified and rested on the ab luminal surface of the basal membrane; they were in mutual contact and characterised by a clear, bloated cytoplasm occupied by few organelles, and contained small bundles of intermediate filaments in the perinuclear area. Lipid droplets of varying sizes were frequently present in the cytoplasmic matrix.

*c. Stromal cells*

The stromal cells surrounding the vessels were adherent to the pericytes, arranged in clusters and filled the interstitial spaces. Although phenotypically diverse, this cell population showed common cytological features and the presence of elements with transitional features. This variability in cytological features can be briefly classified as follows:

1. elements with large hypertransparent cytoplasm, devoid of organelles, having only a cell membrane and a large nucleus in the centre (Fig. 4);
2. elements with an evenly compact cytoplasm, containing in the perinuclear area ergastoplasm with cisterns and an ectasic lumen, Golgi apparatus, peroxisomes, a few lipid droplets and bundles of intermediate (Fig. 5);
3. elements similar to the above but having small, progressively accumulating clusters of osmiophilic material, these being precursors of the lipid droplets (Figs. 6, 7);
4. elements with large cytoplasm devoid of organelles and containing only several lipid droplets (Fig. 8). The stromal cells were generally in mutual contact and bound by desmosomes and hemidesmosomes; in the areas where this cohesion was absent or scanty, the narrow interstitial spaces were found to be occupied by fine fibrils mixed with amorphous material.

**Comment**

The ultrastructural findings herein detailed allow several interesting contributions to current knowledge of haemangioblastomas. The regressive processes in the form of haemorrhagic foci and intra- and extracellular oedema and the formation of micro-macropseudocysts...
can be related to the fragility of small vessels, and in particular to the lack of tight junctions. Although stromal cells show ultrastructural diversification in terms of their cytoplasms, they have the basic morphological features of mesenchymal cells. The ultrastructure of stromal cells was similar to that of the endothelia and pericytes; this confirms the common ancestry of the cell populations of haemangioblastomas and their vasogenic capacity.

Stromal cells can be considered stem cells undergoing even abnormal or incomplete differentiation.
into vascular structures and acquiring a capacity to produce and synthesize lipid material, similar to pre-adipocytes. This capacity is not however limited to stromal cells, but also extends to the endothelia and pericytes. The ultrastructural findings within show both the various stages in the formation of the large lipid droplets, up to their complete occupation of the cytoplasm, and the involvement of several organelles (ergastoplasm, Golgi apparatus, peroxisomes) in generating these deposits. These stages in the lipidization process are similar to those in mesenchymal stem cells which become pre-adipocytes as part of their differentiation process. No cells in any of the cases studied were found to have ultrastructural features attributable either to glial elements or neuroendocrine cells. Only mesenchymal elements were found, whose common histogenesis is also seen by their lipidization capacity not only in stromal cells but also in pericytes and endothelia.

In short, these ultrastructural findings provide the basis for considering haemangioblastoma as a mesenchymal cell neoplasm with a high vasogenic capacity, capable of various differentiations, and in particular of acquiring metaplastic features of pre-adipocytes.

References

Cytology of the oral cavity: a re-evaluation

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Key words
Oral cytology • Oral squamous cell carcinoma • Oral epithelial dysplasia • Oral potentially malignant lesions

Summary
Oral exfoliative cytology, while an economical and practical tool for diagnosis of squamous cell carcinoma and potentially malignant lesions, is not extensively used. The results of conventional (n = 89) and liquid-based (n = 411) oral diagnostic cytology cases are reported and compared to histological diagnosis. Cells were collected using either a Cytobrush device for conventional smears or a dermatological curette (AcuDispo) for liquid-based (Thin Prep) cytology. The “curette technique” allowed for the collection of “accidental” tissue fragments, utilized as microbiopsies. The sensitivity was 86.5% in conventional and 94.7% in liquid-based cytology; specificity was 94.3% and 98.9%, respectively; inadequate samples were present in 12.4% and 8.8% of cases, respectively. Although conventional cytology may be useful in oral squamous cell carcinoma and potentially malignant lesions, liquid-based cytology gives better results, enhances both the sensitivity and specificity, and also provides material for further investigations, e.g. DNA ploidy studies, microhistology, etc.

Introduction
Squamous cell carcinoma (SCC) of the oral cavity and the oropharynx is a frequent malignant neoplasia (6th most frequent cause of cancer-related mortality worldwide). It has been estimated that up to 500,000 new cases of oral and pharyngeal cancer are diagnosed annually and approximately 75% of these occur in the developing world, with a high mortality rate (the Italian Tumour Register indicates 2,978 deaths in 1999, with a 38% overall 5-year survival rate). During the last 25 years no significant improvements have been seen in terms of the survival rate. This is due to the fact that it is usually diagnosed at an advanced stage and that it has a high relapse rate, incidence of second tumours and of metastasis. Moreover, a significant increase in the incidence of oral cancer has been demonstrated in many European countries, such as the UK and France, similar to what has been reported in eastern Europe. Recently, it been demonstrated that screening programmes, based on objective examination of the oral cavity, can reduce the mortality rate for oral SCC, but there are no data for programmes based on other parameters, such as diagnostic cytology.

The fact that both diagnosis of oral SCC and its precursors (dysplasia) are still based almost exclusively on scalpel biopsy is one of the causal factors for the poor prognosis of oral neoplasia, as scalpel biopsy is not only invasive, but also limits sampling to a very restricted area (in particular in the case of multiple lesions) on a relatively small number of sites. Thus, there is an objective risk of insufficient sampling of multiple and/or extended lesions, and it is well known that only one lesion among many, or even a small part of a single lesion, may show a microscopic demonstration of malignancy (carcinoma) or pre-malignancy (dysplasia).

A scalpel biopsy does not usually cover all the potentially malignant oral lesions (PML, i.e. leukoplakia, leuko-erythroplakia, lichen planus, proliferative verrucous leukoplakia, etc.), but is generally limited only to those highly suspect lesions such as erythroplakia, dishomogeneous or verrucous leukoplakia and/or chronic ulcers. Lastly, when a scalpel biopsy is performed on a strongly suspicious lesion the false negative rate may be as high as 23%. It is therefore evident that it would be useful to establish a first level test that could identify oral lesions that, due to morphologic and/or genetic characteristics, should be further investigated with a second level test, as for a Pap test and colposcopy with biopsy for carcinoma of the uterine cervix.

Indeed, it is well known that a Pap test is efficacious in reducing the incidence and mortality rate of cervical carcinoma as it detects intraepithelial pre-neoplastic lesions (dysplastic) before they evolve into invasive
forms (neoplastic). However, although diagnostic oral cytology has been well known for many years, as a simple, non-invasive, painless, inexpensive tool that can be also applied to extensive and multiple lesions, it has not been as widely adopted as cytology for the vaginal cervix. Recent reports\(^5\), also by our team\(^6,7\), indicate that both the efficacy and efficiency of oral cytology may be enhanced by the addition of new techniques that can enhance its sensitivity and specificity. These include computer-assisted cytology\(^5\), liquid-based cytology\(^6,7\), AgNOR\(^6,7\), molecular biology and flow cytometry\(^8,9\).

Computer-assisted cytology is based on the use of dedicated instruments that allow for the identification of both pre-neoplastic and neoplastic lesions with a sensitivity (i.e. the proportion of actual positive cases correctly identified as such) equal to, or higher than that of manual screening, without loss of specificity (i.e. the proportion of correctly identified negative cases).

Liquid-based cytology is a relatively recent method that, to date, has been used mainly for the Pap test, and has given promising results both in terms of the quality of the samples and their adequacy. The analysis of AgNOR (acidic proteins associated with nucleolar regions that are selectively stained by a silver colloid technique) allows for evaluation of the proliferative activity of cells and, therefore, in addition to being a valid prognostic factor in clinical oncology, also allows for detection of dysplastic and/or neoplastic cells in cytology.

To increase the quantity of cells available for cytological examination and other complementary techniques, we established a new sampling technique that was no longer based on the use of a “cytobrush”, but on “scraping” using a dermatological curette. It was immediately evident that, not only did this technique provide more abundant material for cytology and ploidy\(^9,10\), (i.e. the evaluation of the number of homologous sets of chromosomes in cells), but that samples were richer in “accidentally” acquired small fragments that could be used as micro-biopsies, included in paraffin and examined in histology.

The aim of the present investigation was to examine SCC and PML (even those with a low index of suspicion index) by cytology and microhistology, for early identification of cancer or precancerous oral lesions. Innovative methods are now available, such as computer-assisted cytology, liquid-based cytology smears, AgNOR counts and flow cytometry for the study of ploidy, as well as microhistology (histological examination of small tissue fragments recovered with a dermatological curette, forming a “microbiopsy” that can be compared to both histology and conventional cytology).

### Methods

A total of 500 patients referred to the Oral Medicine Section of the Department of Biomedical Sciences and Human Oncology of the University of Turin (Prof. S. Gandolfo, Dr. M. Pentenero and Dr. R. Broccoletti) for scalpel (surgical) biopsy due to the presence of PML or SCC of oral mucosa. Histological examination showed 55 cases of dysplasia (27 low grade and 28 high grade), 100 cases of SCC and 345 cases without morphological evidence of dysplasia or carcinoma. In all cases, cytology was performed, which was conventional (89 cases) or liquid-based (411 cases) (Thin Prep, Cytologic Corporation, Marlborough, MA, USA). A total of 138 cases in this last group had sampling with both Cytobrush and a dermatological curette (AcuDispo Curette, Acuderm inc.); these “micro-biopsies” were processed as traditional “scalpel” biopsies. Computerised cytology with neural networks and AgNOR evaluation (by silver staining according to the Ploton method, measuring the area with a dedicated image analysis system) was carried out in 73 of the 500 cases. Flow cytometry was used in 190 cases to analyse the DNA content in squamous cells on samples in saline solution, using a FACSCalibur flow cytometer (Becton Dickinson).

### Results

A scalpel biopsy, for conventional histological diagnosis, was done in each case. Conventional cytology, performed in 89 cases, demonstrated an 86.5% sensitivity with a specificity of 94.3%. The positive predictive value was 95.7%, with 12.4% of inadequate samples. Computer-assisted cytology, applied in 73 cases, allowed for diagnosis of one case that had initially been classed as negative, increasing the sensitivity rate to 89.0%. Liquid-based cytology, used in 411 cases, had a sensitivity of 94.7% for high grade and carcinoma lesions and a specificity of 98.9%, with a positive predictive value of 95.9% and 8.8% inadequate samples. AgNOR, analysed in 73 cases, demonstrated both a sensitivity and specificity of 100%.

However, there was a high percentage of inadequate samples (15.1%). Ploidy analysis by flow cytometry demonstrated aneuploidy, in 24/40 (60.0%) carcinomas, in 0/6 (0%) verrucous carcinomas, in 16/70 (22.8%) of PML without dysplasia and in 17/30 (56.7%) of PML with dysplasia. Lastly, the “microbiopsies” (138 cases), recovered together with the material used for liquid based cytology, allowed for definitive histological diagnosis in more than 50% of cases\(^10,11\).

### Discussion and conclusions

Conventional exfoliative cytology provides satisfactory diagnostic information. Its sensitivity is higher than the Pap test, with a similar specificity. Computer-assisted cytology gives a slightly higher sensitivity, but the efficiency of this system has not yet been fully demonstrated. In contrast, it would appear that liquid-based cytology is associated with increased diagnostic accuracy of oral cytology thanks to its high sensitivity and specificity. The analysis by AgNOR (even if limited by a high percentage of inadequate samples) was useful in...
further enhancing the sensitivity in dubious cases. The finding of aneuploidy with absence of dysplasia (i.e. epithelial dysplasia not detected by conventional histology), allowed for the identification of lesions that were at risk of evolution.\textsuperscript{8,9} This led to the selection of individuals who required a stricter follow-up regime, with surgical excision of all suspicious oral lesions. Lastly, curette sampling, which covered ample surface areas and/or multiple lesions and provided “microbiopsies”, allowed for a reduction in the number of patients that were required to return for additional work-up and in the quantity of diagnostic (scalpel) biopsies. This leads to a positive cost/benefit ratio for the hospital and less discomfort for patients.\textsuperscript{10-11} Therefore, the advantages offered by this type of sampling, may not apply only to patients referring to hospital structures, but it could be more widely applied to a larger population sample. Indeed, its relative simplicity and “patient friendly” nature make it a good candidate for use in dentistry where most pre-neoplastic and neoplastic lesions are first observed. The adoption of this strategy could, in part, contribute to reducing the percentage of late diagnoses in invasive lesions.

The data obtained in this study enhance present knowledge on the mechanisms of progression of precancerous oral lesions, and suggest that they could be applied to a wider range of precancerous squamous lesions with onset in the head neck region.

References

Benign metastasizing leiomyoma: report of 2 cases and review of the literature

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Key words
Metastasizing leiomyoma • Medical treatment • Lung

Summary
Benign metastasizing leiomyoma (BML) is a benign spindle cell lesion affecting women who have undergone hysterectomy for uterine leiomyomas in young adulthood, and subsequently present pulmonary metastases during the peri-menopausal period. We present 2 cases of BML in women with prior medical history of hysterectomy for multiple myomas. Both patients presented pulmonary metastases at 17 and 12 years after hysterectomy. The pulmonary nodules were totally excised in both cases, and neither patient experienced complications or recurrences after 1 and 2 years of follow up, respectively.

BML is a rare benign entity with a debated pathogenesis. We have developed different hypotheses about its pathogenesis, mechanisms of spread, histological characteristics and commonly employed treatment modalities.

In 1983, multiple smooth muscle lesions in the lungs were classified by Martin who divided them into three categories: metastatic leiomyoma which arise in men and children from an extra-uterine primary source, multiple fibroleiomyomatous hamartomas which consist of multiple leiomyomas of the lung without a primary source, and benign metastasizing leiomyoma (BML) 1. The latter entity is rare. It was first described by Steiner in 1939, and to date less than 100 documented cases have been described in the literature.

We report two cases of benign metastasizing leiomyomas (BML). The first case was observed in a 46-year-old woman who underwent, 17 years previously, a total hysterectomy because of multiple myomas. The patient presented with a long-lasting unproductive cough. The interval between the onset of the symptoms and the consultation was one year. A chest-X ray showed a 3-cm nodule localized in the left upper lung (Fig. 1), and a CT-scan revealed a 23-mm apical mass. A malignant tumour was suspected, and a postero-lateral thoracotomy was performed. Two nodules were discovered during the intervention. Histological findings consisted in a benign mesenchymal proliferation. This lesion was formed of spindle cells with slightly eosinophilic cytoplasms and central fusiform cigar-shaped nuclei with regular outlines and no evident nucleoli, atypia or mitosis (Fig. 2).

Immunohistochemical analysis revealed strong expression of hormonal receptors and actin by tumoural cells (Fig. 3). These findings led to a diagnosis of benign metastasizing leiomyoma of the lung. After 1 year of follow-up, the patient has not had any recurrence.

The second patient is a 52-year-old woman with a past medical history of hypertension and hysterectomy performed 12 years ago. A chest X-ray was performed in a
A preoperative study in relation to a previous cholecystectomy, which showed a diffuse bilateral miliary pattern. A CT scan revealed diffused micro-nodules occupying both lungs without mediastinal adenopathy. Biopsies, which were obtained using micro-thoracotomy, revealed a benign spindle cell proliferation. Thorough examination of the biopsies showed no signs of tumour necrosis, pleomorphic regions or increased cellular atypia. Tumoural cells showed strong expression of oestrogen receptors in about half of neoplastic cells. No expression of progesterone receptors was detected. According to the past medical history of the patient and histological findings, a diagnosis of BML was made. After 2 years of follow up, the patient has not shown any signs of recurrence.

BML affects women who have undergone hysterectomy for uterine leiomyomas in young adulthood and subsequently present pulmonary metastases in the peri-menopausal period. Metastases most commonly affect the lung, but may be located in the spinal cord, heart, peritoneum, retoperitoneum, bone, brain, lymph nodes and skin. Its pathogenesis has been debated for many years. In the past, these lesions were termed ‘multiple fibroleiomyomatous hamartomas’ since they were thought to originate in situ in the lungs according to the presence of entrapped epithelial elements. Smooth muscle neoplasms can in fact develop de novo in virtually any location. However, extra-uterine leiomyomas are oestrogen receptor (ER) negative, and only a low proportion of extra-uterine leiomyosarcomas (13%) show weak and focal oestrogen receptor immunoreactivity. In contrast, most BML are ER positive. Moreover, genomic hybridization, X-chromosome inactivation analyses and the analysis of telomere length have demonstrated a balanced karyotype and an identical X-chromosome inactivation pattern consistent with a monoclonal origin of pulmonary and uterine tumours in one case of BML. Some authors considered these tumours as a misdiagnosed low-grade leiomyosarcoma due to insufficient sampling. A multifocal origin has been advocated in some cases with unusual distribution of metastatic lesions. Still others have suggested that the tumour metastases might have undergone maturation.

Most pathologists now accept these lesions as haematogeneous metastases arising from morphologically benign uterine tumours. Several mechanisms of spread have been theorized. Some authors hypothesized that smooth muscle neoplasms may arise directly from vessel walls, but are associated with extension to the inferior vena cava and does not result in metastatic foci. Others claim that these tumours gain venous access via surgical trauma during hysterectomy. This hypothesis seems to be the most plausible in our two patients. In fact, both women underwent surgical intervention for uterine leiomyomas before the appearance of pulmonary metastases. However, this would not explain visualization of nodules before surgery in some reported cases. The disease process is usually indolent and is generally discovered incidentally. Our second patient was asymptomatic and the pulmonary nodules were discovered fortuitously. In contrast, our first patient was symptomatic. Some authors have reported rapid progression with consequent respiratory failure and death. The radiological appearances of the pulmonary nodules in BML include well-circumscribed solitary or multiple pulmonary nodules ranging in diameter from a few millimeters to several centimeters scattered among the interstitium.

In our first case, a chest X-ray showed a single nodule, while a miliary pattern was observed in our second patient. This pattern is less common, but has been described by Lipton et al. Additionally, there are case reports of BML manifesting as cavitary lung nodules and interstitial lung disease. Endo-bronchial and pleural sparing is characteristic of this disease; while mediastinal and hilar lymphadenopathy is rare. Neither of our patients presented with mediastinal adenomegaly. Nodules have been seen at times ranging from 3 months to 20 years after hysterectomy.
interval that corresponds closely to that seen in our 2 patients. The radiological findings of multiple pulmonary nodules have a broad differential diagnosis, including neoplasms (benign or malignant), vascular lesions, collagen-vascular disease, infectious inflammatory granulomas and non-infectious inflammatory granulomas. Diagnosis is based on pathological findings, and percutaneous fine needle or core biopsy can be used to confirm diagnosis. Histological features show an orderly pattern of intersecting fascicles of acidophilic spindle cells with blunt-ended nuclei without significant cellular pleomorphism or mitotic activity. No malignant features are noted. The histological differential diagnoses are metastasis of leiomyosarcoma, primary pulmonary leiomyomatosis (no history of related gynaecological lesions), lymphangioleiomyomatosis and so-called fibroleiomyomatous hamartoma. Many parameters are used to differentiate leiomyoma from leiomyosarcoma, including tumour necrosis, cell atypia and the mitotic index. However, other parameters can help to determine the true nature of the lesion, including the patient’s age, tumour size, macroscopic findings and the invasion of adjacent structures. In our patients, the histological features were not suggestive of malignancy.

Lymphangioleiomyomatosis is characterized by the proliferation of smooth muscle cells from lymphatic walls in the lung and the lymph nodes. In addition, the immunohistochemical study showed in BML strong expression of both oestrogen and progesterone receptors. This finding led to treatment options based on hormonal manipulation because of its reversibility and the potential to avoid surgical procedures. Medical treatments include long-acting GnRH analogues, progesterone antagonists, and more recently, a selective oestrogen receptor modulator that antagonizes oestrogen action in target tissues. Pulmonary lesions tend to remain stable with occasional regression after treatment. However, therapy may not always be indicated. The effects of natural hormonal changes such as pregnancy and menopause have also been associated with lesion regression. Prognosis of BML is good and depends on the presence of hormonal receptors and the extension of pulmonary nodules. In a study of 10 patients reported by Kayser et al., median survival was 43 months. Many advances have been made in terms of diagnosis, and additional molecular studies could lead to the discovery of markers to identify uterine smooth muscle tumours that have the potential to develop benign metastasizing leiomyomas.

References

Report of a case of intraosseous frontal meningioma

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Key words

Intraosseous meningioma • Skull • Surgery

Summary

Meningiomas without dural connection are rare lesions and often referred to as ectopic. Primary ectopic intraosseous meningiomas are uncommon, accounting for less than 2% of all meningiomas. Herein the authors describe a case of meningioma involving the frontal bone in a 43-year-old man and review the relevant literature.

Introduction

Primary intraosseous meningioma of the skull is a rare tumour often confused preoperatively with a primary bone lesion of the skull. Crawford et al. 1,2 defined true meningioma of the skull as “only those cases in which the dura is clearly not involved or when its involvement is not specifically noted”. They can occur within the subcutaneous tissue of the skin, the orbit, neck, paranasal sinuses, calvaria, salivary glands and along the perineural sheath of cranial nerves. Precontrast computerized tomography with bone windows is often the most helpful imaging technique, and can identify the extent of the bone tumor as well as define the continuity of the inner and outer tables as well as any intracranial involvement. However, the technique is rarely diagnostic, and differential diagnosis includes osteoma and metastatic cancer as well as endocrine or metabolic derangements such as hyperparathyroidism and vitamin A and D hypervitaminosis (in the case of primary hyperostotic or sclerotic lesions) and eosinophilic granuloma, fibrous dysplasia, epidermoid tumor, osteogenic sarcoma, multiple myeloma chondroma, chondrosarcoma and giant-cell tumor (for osteolytic lesions). We herein describe the case of an intraosseous frontal meningioma occurring in a 43-year-old man and discuss its clinical and pathological features in light of previously described cases in the literature.

Case report

A 43-year-old man was referred to our Department for evaluation of transient right-sided headaches with a progressively enlarging hard and painless mass over the right frontal area that had been present for 4 years. Routine blood investigations were within normal limits and neurological examination revealed no neurological deficits; brain contrast CT scan showed a right osteolytic lesion involving the diploic layer in the frontal bone without intracranial or dural enhancing (Fig. 1). The patient underwent surgery with a pterional modified skin flap and a right frontal craniotomy with complete resection of the lesion which measured approximately 3 x 3 cm (Fig. 2) following acrylic cranioplasty. Intraoperatively, no dural infiltration was noted, but it was however coagulated with the use of bipolar forceps. The postoperative period was uneventful and pathologic findings were consistent with a WHO grade I meningioma (Fig. 3). MRI performed 6 months later revealed no signs of recurrence.

Discussion

Hoye et al. 3 classified meningiomas occurring outside the cranium into four groups: 1) primary intracranial tumors with direct extracranial extension; 2) tumors originating from arachnoid cell rest, occurring within the sheaths of cranial nerve; 3) tumors occurring extracranially without any apparent connection with the

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foramina of cranial nerves (these lesions presumably arise from ectopic rests of arachnoid cells); 4) benign-appearing intracranial meningiomas with extracranial metastases. In this report, the case falls into the third type of meningioma.

Several pathogenic mechanisms have been proposed for primary ectopic meningiomas. Azar-Kia et al. suggested that arachnoid cap cells become trapped in the cranial suture during birth, while Shuangshoti et al. attributed them to pluripotent mesenchymal cells that are transformed into neoplastic cells by an unknown trigger. A third explanation is that the lesions are secondary to previous trauma (Cushing and Eisenhardt’s theory).

As noted by Rosahl et al., only 16 cases of primary osteolytic intraosseous meningioma have been reported, and histological examination classified these tumors as meninogotheliomatous (62.1%), transitional (18.2%), fibroblastic (6.1%), psammomatous (6.1%) and malignant meningioma (7.6%).

Conclusion

Primary intraosseous meningiomas are rarely correctly diagnosed preoperatively and are usually mistaken for primary bone tumors. The clinical features and prognosis of this rare lesion are not different from common meningiomas, and surgical intervention is a valid therapeutic approach.

References

5. Shuangshoti S. Primary meningiomas outside the central nervous
Undifferentiated gastric carcinoma with lymphoid stroma (lymphoepithelioma-like carcinoma/medullary carcinoma)

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Key words
Lymphoepithelioma-like carcinoma • Epstein-Barr virus • Gastric carcinoma

Summary
Undifferentiated gastric carcinoma with lymphoid stroma is a histological type of gastric cancer with favourable prognosis, microscopically characterised by nests of neoplastic epithelial cells intermingled with a dense lymphoid proliferation. Various studies have shown a close relationship between undifferentiated gastric carcinoma with lymphoid stroma and Epstein-Barr virus infection; the presence of viral DNA in tumour cell nuclei has been demonstrated using polymerase chain reaction and Epstein-Barr virus-encoded small RNA in neoplastic cell nuclei have been found using in situ hybridization. We describe two cases of undifferentiated gastric carcinoma with lymphoid stroma, one infiltrating the submucosa of the gastric body and the other invading the muscularis propria of the antrum. No lymph node neoplastic invasion was documented in either case. Epstein-Barr virus was detected in the neoplastic cell nuclei in both cases with in situ hybridization.

Introduction
Undifferentiated gastric carcinoma with lymphoid stroma (medullary gastric carcinoma or gastric lymphoepithelioma-like carcinoma) is a histological type of gastric cancer with favourable prognosis, microscopically characterized by nests of neoplastic epithelial cells intermingled with a dense lymphoid proliferation analogous to proper lymphoepitheliomas, which originate in other sites, e.g. salivary glands or rhinopharynx.
A male predominance (male/female ratio about 3:1) and a low frequency of lymph node invasion have been reported. Moreover, various studies have shown a close relationship between undifferentiated gastric carcinoma with lymphoid stroma and Epstein-Barr virus (EBV) infection. The presence of viral DNA in tumor cell nuclei has been demonstrated using polymerase chain reaction (PCR), and by in situ hybridization the presence of EBV-encoded small RNA (EBER) in neoplastic cell nuclei has also been documented, but not in either normal gastric epithelium or lymphoid cells.

Histopathological findings
In both cases nests of neoplastic epithelial cells not forming glands, intermingled with a dense lymphoid infiltrate, composed of mature B and T lymphocytes, were found (Fig. 1 a, b, c and d). In case 1, the tumour infiltrated the submucosa (early gastric cancer; type of...
growth according Kodama: PEN A), whereas in case 2 an advanced carcinoma invading the muscularis propria was found (T2a according to AJCC, 2006). No lymph node involvement was found in either case. Using in situ hybridization, EBV was detected in neoplastic cell nuclei (Fig. 2), but not in normal mucosa and lymphoid cells.

Discussion

Various studies have reported the characteristic clinicopathological aspects of undifferentiated gastric carcinoma with lymphoid stroma, such as male predominance and a similar prevalence of intestinal and diffuse type. One of our two cases refers to a man with a cancer originating in the body of the stomach, whereas the other originated in the antrum of a female patient. Some studies have reported that EBV-positive gastric carcinoma arises more often in the cardia and middle part of the stomach, while others have found that the antrum is the most frequent site of origin. In accordance with various studies, in both our cases, no lymph node invasion was found. These two cases also had EBV as demonstrated by in situ hybridization-EBER in tumour cell nuclei, but not in normal and lymphoid cells.

Epstein-Barr virus (EBV) is an oncovirus showing lymphtropism as well as epitheliotropism. Its genome
has been found in various malignant tumours, such as Burkitt’s lymphoma, nasopharyngeal carcinoma, Hodgkin’s disease, in most undifferentiated gastric carcinoma with lymphoid stroma, and in 5%-10% of conventional gastric adenocarcinomas. An aetiological association between EBV and undifferentiated gastric carcinoma with lymphoid stroma has been hypothesized on the basis of the presence of EBV DNA in most cancer cells, and its absence in the neighbouring normal epithelium and in lymphoid cells intermingled with the tumour, as in our cases. EBV DNA extracted from cancer cells has been reported as monoclonal, following hybridization of EBV terminal repeat fragment by Southern blot; moreover, high serum anti-EBV antibody levels have been found many years before diagnosis of EBV-positive gastric cancers.

In situ hybridization-EBER positivity is considered a marker of a latent EBV infection, since it is found at high levels (10^4-10^6 copies/cell) in cells infected by EBV without any clinical signs of infection. For this reason, we believe that EBV infection in cancer cells is in a latent condition, and the exact role of EBV infection in gastric carcinogenesis remains to be elucidated.

References

3. Burke AP, Yen TS, Shekitka KM, Sobin LH. EBV DNA extracted from cancer cells has been reintermingled with the tumour. In situ hybridization-EBER positivity is considered a marker of a latent EBV infection, since it is found at high levels (10^4-10^6 copies/cell) in cells infected by EBV without any clinical signs of infection. For this reason, we believe that EBV infection in cancer cells is in a latent condition, and the exact role of EBV infection in gastric carcinogenesis remains to be elucidated.
Primary cutaneous marginal zone B-cell lymphoma: clinical and histological aspects

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Key words
Primary cutaneous marginal zone B-cell lymphoma

Summary
According to the WHO-EORTC classification of cutaneous lymphomas, primary cutaneous marginal zone B-cell lymphoma are now well characterized. We report here a case of primary cutaneous marginal zone B-cell lymphoma in a 51 year-old man in which the diagnosis was made using both histology and immunopathology. The patient had no remarkable medical history, no history of either acute inflammation or insect bite, and presented with a 5 cm solitary asymptomatic erythematous firm, multinodular and infiltrated plaque on the back for 12 months. Histological examination and immunohistochemical study of a cutaneous biopsy provided a differential diagnosis between B cell lymphoma and lymphocytoma cutis. Full body work up revealed no signs of extracutaneous dissemination. The patient underwent surgical excision of the nodule. Histological examination showed a histological and immunophenotyping profile typical of primary cutaneous marginal zone B-cell lymphoma. The lesion was completely excised with clear margins and no recurrence occurred after a 12 month-follow-up period. Primary cutaneous marginal zone B-cell lymphoma are low-grade lymphomas that have an indolent course and a high tendency to recur. They should be differentiated from lymphocytoma cutis and from the other types of cutaneous B cell lymphomas that have a different course and prognosis.

Background
Primary cutaneous marginal zone B-cell lymphomas (PCMZL) are low-grade lymphomas that originate in the skin, with no evidence of extra-cutaneous disease. We report, a case of PCMZL in a 51 year-old man. We further highlight the precious contribution of histology and immunopathology in the diagnosis of PCMZL. The difficult differential diagnosis includes lymphocytoma cutis or other types of B-cell lymphomas, especially follicle centre lymphoma.

Case report
A 51-year-old Tunisian male with no remarkable medical history and no history of either acute inflammation or insect bite presented with a solitary nodule of his back for 12 months. Cutaneous examination showed an asymptomatic erythematous solitary firm, multinodular and infiltrated plaque of the left side of the back 5 cm in diameter (Fig. 1). Physical examination showed no lymphadenopathy and no hepatosplenomegaly. Histological examination and immunohistochemical study on a cutaneous biopsy gave a differential diagnosis between B cell lymphoma and lymphocytoma cutis. Oto-rhino-laryngological examination, routine laboratory analyses and lymphocytoma cutis. Full body work up revealed no signs of extracutaneous dissemination. The patient underwent surgical excision of the nodule. Histological examination showed a histological and immunophenotyping profile typical of primary cutaneous marginal zone B-cell lymphoma. The lesion was completely excised with clear margins and no recurrence occurred after a 12 month-follow-up period. Primary cutaneous marginal zone B-cell lymphoma are low-grade lymphomas that have an indolent course and a high tendency to recur. They should be differentiated from lymphocytoma cutis and from the other types of cutaneous B cell lymphomas that have a different course and prognosis.

Fig. 1. 5 cm erythematous solitary multinodular plaque of the left side of the back.

Acknowledgment
We thank Professor J. Wechsler who kindly contributed to the definitive diagnosis.

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and radiological explorations were normal, as was bone marrow biopsy. Borrelia IgM and IgG serology was negative. The patient underwent surgical excision of the nodule. Histological examination showed a diffuse infiltrate of the dermis and the hypodermis sparing the epidermis, superficial dermis and the adnexal structures (Fig. 2). There were reactive follicles with a distinct mantle zone in the reticular dermis. The infiltrates were composed of small lymphocytes (centrocyte-like cells), lymphoplasmacytoid cells and plasma cells (Fig. 3). Immunohistochemical studies revealed that small lymphocytes were diffusely positive for CD20, CD79a and Bcl2 (Fig. 4) and negative for CD10 and Bcl6. Reactive germinal centers were negative for Bcl2 and CD21. Lymphoplasmacytoid and plasma cells in the superficial dermis and at the periphery of the infiltrate expressed a monotypic cytoplasmic immunoglobulin lambda light chain. We also observed scattered reactive T cells, positive for CD3. The correlation of histological features with the immunophenotyping profile led to a diagnosis of PCMZL. The tumour was completely excised with clear margins. There was no recurrence of the lesion after a 12 month-follow-up-period.

Discussion

According to the WHO-EORTC classification, PCMZL are indolent lymphomas composed of small B cells, including marginal zone (centrocyte-like) cells, lymphoplasmacytoid cells and plasma cells. They account for only 2%-16% of all cutaneous lymphomas and are the second most common after follicle center lymphomas. Senff et al. reclassified 300 patients with primary cutaneous B-cell lymphomas according to the new WHO-EORTC classification and found that 23.6% of patients had PCMZL. They occur most frequently in adults with an average age of 55 years and seem to have a male predilection. Clinically, they frequently present as red to violaceous solitary papules, plaques or nodules with a deeper infiltration, as an “iceberg”, than thought by palpation. The trunk is the most involved region,
followed by the upper limbs, face and neck and less frequently the lower limbs. In 41.7% of cases, they present as multiple cutaneous lesions with either a regional (24.5%) or disseminated involvement (17.2%)\(^4\). Extracutaneous dissemination is very rare (6.2%), but should be always considered. Histology and immunophenotyping are the gold standard for correct diagnosis. Our case showed histological and immunophenotyping features typical of PCMZL\(^7\). Diagnosis was not obvious by cutaneous biopsy, since it was differential with lymphocytoma cutis. Microscopically, marginal zone B-cell lymphomas (MZCL) are characterized by dense lymphocytic infiltrates mostly distributed on the reticular dermis and often extending to the hypodermis (bottom heavy). There was no epidermotropism which always has a spared papillary dermis forming a Grenz zone. As stated by Servitje et al., the infiltrate can be limited to the superficial reticular dermis in a so-called top-heavy pattern. The infiltration can be arranged as a nodular or less frequently diffuse pattern (as in our patient) with frequent adnexal involvement\(^7\). 

Cytologically, MZCL are characterized by a polymorphous infiltrate that included small- to medium-sized lymphocytes with dense chromatin and a pale cytoplasm (marginal zone cells) with frequent reniform nuclei (B-monocytoid cells) and a variable proportion of sparse larger centroblast-like cells (blastoid cells) intermingled within the infiltrate. As demonstrated in our case, germinal centre-like structures, surrounded by neoplastic cells mimicking incomplete or aberrant mantle zones, are frequently observed. These germinal centres are considered as reactive and were often colonized by the neoplastic infiltrate. In most cases, there were areas of plasmacytoid differentiation with the presence of mature plasma cells that were constantly disposed at the periphery of the infiltrate and under the dermo-epidermal junction. Marginal zone B-cells were CD20+, CD79a+, CD5- and CD10-. Neoplastic cells were bcl2+. Servitje et al. stated that usually a low number of large CD30+ cells are present in the infiltrate. A significant number of reactive T cells (as in our case) expressing the CD3 phenotype are also frequently present in MZCL. They can be so numerous that they mask the neoplastic population.

Special attention must be taken in differentiating MZCL with reactive germinal centre from follicle center cell lymphomas. Indeed, reactive germinal centre cells in MZCL are negative for bcl2\(^7\). Furthermore, follicle center cell lymphomas usually express CD10 and bcl6 antigens whereas MZCL does not. In our case, histological examination of the biopsy specimen was unable to differentiate between lymphocytoma cutis and B cell lymphoma, but the excised tumour expressed a monotypic cytoplasmic immunoglobulin light chain type Lambda, thus eliminating a diagnosis of lymphocytoma cutis. 

Patients with solitary lesions can be effectively treated with radiotherapy or excision. In regional or disseminated involvement, radiotherapy and chemotherapy are suitable therapeutic options\(^10\). Complete clinical response ratio with surgery and radiotherapy approaches 100%, although it may be lower with chemotherapy\(^7\). PCMZL have an indolent course but have a high tendency to recur. In the largest reported series, the relapse rate after surgery and radiotherapy is, respectively, 31.6% and 46.9%\(^4\). Recurrence can be localized at the treated site or at a distant cutaneous site, even at long times following initial treatment. Two or more relapses can occur at a significant incidence\(^7\). Extracutaneous dissemination is very rare and ranges from 4% to 6%\(^7\).

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C1-C5 in breast cytology
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The detection of breast lesions with clinical or instrumental investigations requires definition of the nature of such lesions. Some cases can be managed only with clinical support or radiological imaging, although dubious or suspicious cases need morphological analysis with cytology and/or histology. Fine needle aspiration (FNA) is adopted in our clinic as a first choice because of its low invasiveness and costs. We perform FNA in cooperation with a radiologist under ultrasound guidance, to ensure perfect targeting of lesions, spreading of cytologic material on smears and immediate evaluation of their adequacy so that the exam can be repeated in the case of scant cellularity.

Regarding FNA reporting, for each nodule, a form containing the main anamnesis, clinical and radiological information is used to better interpret the lesion; the final cytological diagnosis is reported using one of the five diagnostic categories (Inadequate: C1; Benign: C2; Atypia probably benign: C3; Suspicious of malignancy: C4; Malignant: C5) as proposed by the NHS Breast Screening Programme (NHSBSP).

Using these cytologic categories allows a clear dialogue with clinicians, makes easy to address patients to different clinical pathways (surgery or follow-up) and also provides the possibility of cyto-radiological, and cyto-histological comparison. The categories cover the main cytological morphological criteria to correctly identify all types of breast lesions from clearly benign to clearly malignant, in addition to reactive and inflammatory lesions (i.e. intramammary lymph nodes, inflammations, etc.) with particular attention towards differential diagnosis. Dubious and difficult cases for which the pathologist cannot report a definitive diagnosis are also thoroughly treated. A large variety of smears coming from our routine files related to the breast screening programme will be shown.

Breast cytology plays an important role as a second level exam for mammographic screening programme that started in our region (Friuli Venezia Giulia) in 2006 for women between 50 and 69 years of age. During the first screening round (2006-2007), 341 women were found to have radiological abnormalities that needed further morphological exams, and 259 (76%) of these underwent FNAC. 307 nodules were detected and we observed the following cytological results: 47.6% of nodules were malignant (C5), 30.9% were benign (C2); 9.5% of them were reported as C3 and 7.8% as C4. Inadequate samples (C1) represented only 4.2%.

Cytological results were compared either with history or clinical follow-up; all malignant cases (C5) were confirmed by histology; benign cases (C2) were clinically confirmed by follow-up (at least 6 months) or in some rare cases by histology.

This high level of cooperation between the cytopathologist and radiologist has reduced the inadequacy rates to low percentages and increased the number of cases with definitive diagnosis (benign; malignant), leading to a reduction of diagnostic surgical biopsies and frozen sections.

Good quality cytology allows reduction of number of inconclusive diagnoses (Suspicious Rate: C3+C4) to percentages lower than 20% as recommended by the NHSBSP (1993); in our screening-related experience (2006-2007), we observed a suspicious rates of 17.3%. We believe that the important role played by FNAC under ultrasound guidance in diagnostic breast cytology can be only accomplished by daily cooperation among pathologists, radiologists and clinicians (Breast Unit); this will provide better results not only for a screening-related programme where it is important to respect quality indicators, but also for all the different applications of cytology in daily routine.

Endometrial Cytology: up-take during hysteroscopy
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Introduction. Abnormal uterine bleeding is the most frequent reason for gynaecological consultation. In menopause, it is associated with endometrial carcinoma (8%-10%) and allows diagnosis in early stages in 70% of cases.

To date there is no screening protocols for patients with asymptomatic adenocarcinoma as there are non-invasive diagnostic exams with good sensitivity and specificity. Thus, a minimally-invasive procedure that assures accurate diagnosis would be welcome. Conventional cytology (washing, brushing) is not indicated in asymptomatic patients due to the unfavourable cost-benefit ratio, the low sensitivity and specificity of current methods and the results can also be compromised by the haematic contamination of slides, with difficulty in diagnosis. The aim of this study was to optimize technical procedures, using the cytologic material collected during hysteroscopy performed in the Bari Hospital.

Materials and methods. 88 patients with an age between 28 and 81 years were studied; the women had a history of adenocarcinoma, and therapy with tamoxifen. All women were submitted to hysteroscopy, with irrigation of physiologic solution used to distend the uterine cavity; the fluid was subsequently used to collect cytologic material. The liquid was treated as follows:
- centrifuged (1500 rpm x 10 minutes);
- incubated pellet with CytoLyt (15 minutes);
- centrifuged (1500 rpm x 10 minutes);
- pellet in PreserveCyt non-gyn;
- ThinPrep 2000;
- Pap staining.

The cytological specimen was compared to the histological specimen in those cases where a histological biopsy was available.

Results. Cytologic exam of the 29 patients with a clinical and hysteroscopic history of adenocarcinoma led to 23 positive diagnoses for neoplasia and 6 non-diagnostic; in 18 cases, histologic exam confirmed the neoplasia. The cytological pattern gave the following diagnoses in the 20 patients in therapy with tamoxifen (hysteroscopy: atrophic and/or cystic endometrium): 4 positive for neoplasia, 10 negative for neoplasia, 6 inadequate; biopsy was performed in 12 patients, with confirmation of neoplasia in only one case, while in the remaining 11 patients atrophic endometrium was diagnosed. In our study, we considered an additional 39 patients with different symptoms (menopause, simple and complex hyperplasia, dys trophy) with a hysteroscopic picture of proliferative, atrophic or malignant endometrium. For these subjects, we studied the
cytological liquid: 6 were positive for neoplasia, 26 negative for neoplasia, 3 inadequate for diagnosis; a biopsy was taken in 30 cases with a diagnosis of adenocarcinoma in two cases, while in remaining cases the diagnosis was benign.

Conclusions. Cytology with ThinPrep facilitates diagnosis and management of endometrial pathology, with minimal invasion. The procedure is well accepted by the patient, and has the potential to become the first choice for diagnosis of endometrial carcinoma with a minimal percentage of inadequate samples. It is also useful for making diagnosis on atrophic mucosa and on mucosa stressed by tamoxifen in which obtaining a biopsy sample is difficult.

The SIAPEC/IAP classification system of thyroid nodules by fine-needle cytology

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Introduction. Fine-needle cytology (FNC) is the most accurate and cost-effective method for diagnosis of thyroid lesions. Its diagnostic efficacy approaches 100% in the classification of benign and malignant lesions, in agreement with histology, which represents the “gold standard”. However, some follicular-structured lesions, histologically corresponding to follicular neoplasms and including follicular carcinoma and the follicular variant of papillary carcinoma, cannot be correctly identified using FNC. In this setting, the technique can help to distinguish patients who are candidates for surgical intervention from those who should only be followed-up. The cytopathologic criteria for including a thyroid lesion submitted to fine needle aspiration (FNA) in either category, and the number of categories each with a different clinical strategy, are still a matter of controversy.

History of the classification systems. The first classification system for cytology was devised by Papanicolau in 1945 who classified the results of the Pap test (cervical cytology) into five classes: normal, infectious, indeterminate (class III), dysplastic, and malignant. This classification has represented the cornerstone for all successive systems and was replaced in 1991 with the Bethesda reporting system. Lowhagen adopted a similar classification system for fine-needle aspiration cytology. He identified three categories: benign, suspicious, and malignant. This classification has represented the “gold standard”. However, some follicular-structured lesions, histologically corresponding to follicular neoplasms and including follicular carcinoma and the follicular variant of papillary carcinoma, cannot be correctly identified using FNC. In this setting, the technique can help to distinguish patients who are candidates for surgical intervention from those who should only be followed-up. The cytopathologic criteria for including a thyroid lesion submitted to fine needle aspiration (FNA) in either category, and the number of categories each with a different clinical strategy, are still a matter of controversy.

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variant of papillary carcinoma. In these cases, only histology (and not cytology alone) can provide definitive diagnosis. This category accounts approximately for 20% of the cytologic reports.

About 80% of TIR 3 diagnoses are benign lesions, whereas only 20% are malignant tumours after histologic examination. Some immunohistochemical markers such as Galectin-3, HBME-1, Cytokeratin 19 may improve the accuracy of cytologic diagnosis. Although they do not have a well-established predictive value, they can be used following strict diagnostic protocols to discriminate positive cases (which benefit from the surgical option) from negative ones (follow-up).

Some cases where the cytologic changes are too mild to be included in the TIR 4 category, but too marked to be included in the benign category (TIR 2), can be classified as TIR 3. The choice of whether or not to include these samples in the “low risk” category must be supported by an adequate description in the medical report.

Surgical excision of the lesion and histological examination is recommended. Intraoperative histological examination is not recommended. The surgical option should be evaluated in the clinical and imaging setting.

TIR 4. Suspicious of malignancy. This class represents a heterogeneous group of lesions, and includes samples without a sufficient amount of malignant cells or without cytological atypia sufficient for diagnosis of cancer. This category almost always includes suspicious papillary carcinomas and accounts for about 5% of cytological diagnoses.

It is recommended that FNC be repeated, according to the clinician’s or cytopathologist’s discretion. Surgery with intraoperative histological examination is recommended.

TIR 5. Diagnostic of malignancy. All cases with a diagnosis of malignant neoplasm (papillary, medullary and anaplastic carcinomas, lymphomas and metastasis) are included in this category. It accounts for 5%-15% of cytologic diagnoses. The medical report should contain an adequate cytologic description.

Surgery for differentiated carcinomas is recommended. The surgical option should be evaluated in the clinical setting and on the basis of the cytologic report.

FNC is a screening test. A definitive diagnosis can be made only after histologic examination. For anaplastic carcinomas, lymphomas and metastatic lesions, the continuation of the appropriate diagnostic procedure is recommended.

The American Thyroid Association (2006) refers to a four-tiered classification taken from the most common cytologic reports and where only the “indeterminate cytology” category, among the FP, is specifically identified; the other three are “nondiagnostic aspirates”, “aspirates suggesting malignancy” (corresponding to the Thy 5 of the BTA) and “benign cytology”. It should be noted that in the ATA Guidelines Taskforce the category of cytopathologists is not mentioned. The Papinicolau Society of Cytopathology, during its 2006 USCAP Meeting in Atlanta (GA) and the NCI Meeting (2007) added, with respect to the BTA classification, a new category which is placed between “Benign, non-neoplastic” and “Follicular Neoplasm”. This new category is called “Cellular lesion, cannot rule out Follicular Neoplasm”.

The clinical significance of a classification system for cytology relies on the reproducibility of morphologic criteria and the simplicity of its application to routine practice. Morphologic criteria are well established for all categories except for “follicular proliferation/lesion” (indeterminate or Thy 3 of the BTA classification). This category has been the subject of many papers, and is the cornerstone for the correct application of all classification systems. In fact, cell morphology may overlap with the category of non-neoplastic lesions (Thy 2 of the BTA classification) and, on the other extremity, may show features in common with the follicular variant of papillary carcinoma. Although many studies have attempted to identify rigorous criteria for discriminating totally benign nodules from malignant follicular neoplasms, a variable proportion of non-neoplastic lesions are still inappropriately referred for surgery. Thus, a classification system should harmonize a reasonable number of categories with well-identified treatment strategies and sufficiently accepted cytologic criteria for each category. To ensure the clinical applicability of all classification systems, it is necessary to draw limits for each category based on literature reports. This is especially important for the categories “follicular proliferation” (Thy 3) and “suspicious for malignant neoplasm” (Thy 4) since they are particularly subject to become a ‘wastebasket’ for difficult cases. In this setting, the inadequate category (Thy 1) should not exceed 20% of all diagnoses, the “non-neoplastic lesion” category should range between 55%-70% of cases, the “malignant neoplasm” (Thy 5) is usually below 5% and the “suspicious” category (which encompasses “follicular proliferation/lesion”, Thy 3, and “suspicious for malignancy”, Thy 4) should not exceed 25% of all diagnoses.

Future developments. Better discrimination between the above-mentioned categories may emerge from future studies on the immunocytochemical and molecular expression of “malignancy markers”. The immunocytochemical positivity of HBME-1, Galectin-3, Cytokeratin 19, Ret proto-oncogene, Retinoblastoma-1, and Thyperoxidase in neoplastic thyrocyes and the molecular detection of mutations in the Ret, BRAF-1, K-Ras and PPAR-gamma genes may represent innovative resources for identifying malignant differentiated neoplasms. These techniques can be also applied to specimens collected in a liquid solution (LBC: liquid-based cytology or TLC: thin-layer cytology), which allows the utilization of residual cells for additional investigations after morphologic diagnosis.

References


Cooper DS; the ATA Guidelines Taskforce. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2006;16:1-33.

Elsheikh TM. Follicular lesions of the thyroid: classification and criteria. Meeting of The Papinicolau Society of Cytopathology, USCAP Atlanta, February 11-17, 2006.


Ylagan LR, Farkas T, Dehner LP. Fine needle aspiration of the thyroid: a cytohistologic correlation and study of discrepant cases. Thyroid 2004;14:35-41.

Working Group SIAPEC-IAP for the Classification of Thyroid Cytology 2007 www.siapec.it.
Renal metastasis of malignant myoepithelioma of the breast. Cytologic diagnosis by fine needle aspiration biopsy

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Malignant myoepithelioma (MM) is a rare tumour commonly occurring in salivary glands and breast. The literature has described only a few cases that were associated with distant metastases after several months or even years after the first diagnosis. Describe a case of breast MM metastasizing to the kidney and lung two years after the first diagnosis.

Case report. Female patient, 75 years old, in whom upon a CT chest investigation presented a thickening of the right pulmonary lower lobe with a maximum diameter of about 10 cm, involving the pleura, with necrotic areas and extrinsic compression of the bronchi bronchial tree, highlighted by fibrobroncoscopy; an abdominal CT survey also detected a neoformation in the right kidney about 2 cm in diameter. The patient reported a prior breast quadrantectomy two years earlier at another hospital with histological diagnosis of MM of the breast staging (PT1 N0) established by the negativity of the sentinel lymph node. At the radiology unit of our structure, two biopsy samples were collected under CT guidance: the first using a shearing needle (18 G) on a lung lesion and a second sample using a fine needle (23 G) on the renal lesion. The renal lesion was aspirated twice: the first was inadequate (acellular sample), while the second contained large amounts of cells. The material was placed on a slide and after washing the needle a cell block was obtained for additional investigations. A slide strip was left to air-dry and then directly stained with rapid May-Grunewald-Giemsa; microscopic observation showed cells in a quality and quantity sufficient for diagnosis (Fig. 1), and the biopsy was therefore considered “adequate”. Two other slides were prepared, fixed with ethanol and colored at a later time in the laboratory with Papanicolaou stain.

Final microscopic analysis showed a sample consisting of individual cells in clusters or sometimes three-dimensional that were predominantly spindle-shaped (Fig. 2) and sometimes polygonal (Fig. 3), with microvacuoles and a clear cytoplasm. The nuclear shape was oval with finely granular chromatin and unremarkable nucleoli, in the absence of evident mitosis. Hyaline amorphous material was noted in a necrotic and haemorrhagic background.

Discussion. MM are extremely rare and difficult to identify from a morphological view of differential diagnosis including such as spindle cell sarcomas, fibromatosis, some myofibroblastic lesions, and metastatic epithelial tumors. Therefore, it is essential to know the clinical history and utilize additional methods such as immunocytochemistry for proper diagnosis. In the present case, immunocytochemical investigations gave the following results: Vimentin (+); Actin-smooth muscle (+) (Fig. 4), GFAP (-), S100 (-), which is consistent with results described in the literature. Kidney metastases are rare. The correct cyto-morphological definition of these lesions requires precise classification of clinical and
radiological lesions, as secondary renal lesions often occur as individual nodules in the same way as primary tumors 3.

**Human papilloma virus genotyping using direct sequencing of L1 region in low-grade squamous intraepithelial lesions**

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One of the major problems in screening programs for carcinoma of the cervix is the treatment of low grade squamous intraepithelial lesions (SIL). It is well known that, according to the Bethesda System, the presence of koilocytes in PAP smears allows a diagnosis of low grade SIL. On occasion, cervical biopsies from patients with a diagnosis of SIL fail to show definite dysplastic changes with the presence of bona fide koilocytes being the only abnormality. Additional technologies have been used to overcome this problem such as immunohistochemistry using antibodies anti-HPV and p16, in situ hybridization with specific HPV-probes, hybrid capture 2 test on liquid based cytology, and MY09/MY11 1 or GP5+/GP6+ PCR 2.

We evaluated a series of cases of low and high grade CIN from formalin fixed paraffin embedded tissues. Most of the cases with a 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 3. Negative cases were tested by E6/E7 PCR using primers described by Sasagawa et al. 4.

We found that biopsies from cases from 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 the virus. In particular, among 55 cases studied 44 (80%) were negative, while 11 (20%) showed HPV infection. After sequencing, HPV 17 was found in 2 cases, HPV 6, 11, 15, 34, 70, 74 in one case each, 2 cases showed multiple infections and in one case HPV was undetectable. These preliminary results suggest that genotyping of HPV virus may play a role in distinguishing a subset of patients in whom strict follow-up and not aggressive treatment is warranted.

**References**


**The contribution of cytology to neoplastic effusions**


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The accumulation of fluid in coelomic body cavities may be due to hydrostatic factors, inflammatory processes or malignant tumors. Detection of neoplastic cells in effusions is extremely important as it may give information regarding the primary tumor site, when unknown or for cancer staging and for cancer recurrence in the follow-up of oncologic patients. A review of our data from the last five years showed that of 3000 cases, 21.9% of pleural effusions and 20.7% of peritoneal effusions were positive for neoplastic cells. Cytological diagnosis of malignancy had been made in 32.7% of positive pleural effusions and in 35.5% of peritoneal effusions. Cytology has an important role in the detection of neoplasms and for suggesting the primary tumor sites, which can complement additional diagnostic techniques such as lab exams, CT, MRI, PET, and ultrasound.

The case we report concerns a 65-year-old man, non-smoker, former art teacher; artist, and painter without any known professional risk factor. The patient was admitted to the pneumology department complaining of thoracic pain that increased by inspiration, and for slight respiratory difficulties augmented by physical efforts, which had been progressively worsening. A chest X-ray showed right basal pleural effusion; and a blood test showed D-dimer positivity; the patient was hospitalized for a suspect pulmonary embolism. Anglo CT was negative, and scintigraphy showed only a possible pulmonary microembolism. After starting anticoagulant therapy, the clinical condition improved and he was discharged from hospital. When the patient returned for a control visit, the persistence of pleural effusion was noted and he was given a pleural tap; the fluid was submitted for cytological analysis.

Cytology detected atypical mesothelial cells, mostly isolated with dysmorphic nuclei and scanty cytoplasm and an inflammatory background; the small amount of fluid submitted did not allow preparation of a cell block, and thus definitive diagnosis was not possible. The patient was contacted and, as pleural ef-
fusion was still present, he was given another pleural tap; the pleural fluid was then submitted for cytologic analysis. This time there was a greater amount of material available, which allowed cell block preparation. Morphological observation revealed the same elements previously detected. Immunohistochemistry (Calretinin; EMA; Desmin) was performed with the following results: Calretinin was positive (nuclear and cytoplasmic positivity); EMA showed membrane positivity; Desmin was negative in atypical mesothelial cells and positive in active hyperplastic mesothelial cells.

A diagnosis of suspicion for mesothelioma was made despite the scarce clinical and instrumental evidence and the absence of a work history to asbestos exposure.

The patient underwent pleuroscopy, which was negative, but in consideration of the strong cytologic suspicion, random pleural biopsies were collected which showed the presence of an initial well differentiated pleural mesothelioma, also confirmed by the following pleural decortication.

According to our data records, 38.2% of pleural effusions positive for malignancies in men are due to pulmonary neoplasms and 30.2% are due to mesotheliomas; the incidence of the latter appears to be increasing.

Malignant mesothelioma is a rare tumor; but it has become an important diagnostic, social and epidemiologic issue since it has a demonstrated relationship with asbestos exposure (professional and not).

Histologic diagnosis of mesothelioma can be rather difficult, although several classifications and different morphologic criteria have been suggested; if diagnosis is difficult on histology, it is even more challenging on cytology. Pleural reaction to any kind of pathologic injury is usually represented by an increase of pleural fluid and by recruiting different inflammatory cells such as lymphocytes, granulocytes or histiocytes depending on the type of injury.

Irritative injury also involves mesothelial elements that become activated, and are sometimes hardly distinguishable from neoplastic cells. For this reason, cytologic diagnosis of mesothelioma is difficult and the only morphology, when it is also associated with clinical data, may not be sufficient for definitive diagnosis. Immunohistochemistry, especially when performed on cell blocks, is an important diagnostic tool in those cases for which morphology may not be conclusive.

For cytologic examination of effusions, it is not possible to establish a definitive immunohistochemistry protocol; this is because cytology must be based on study of cellular morphology, as well as patient gender and clinical and epidemiologic data; only in a second phase cytology can be supported by immunohistochemistry, which is of great help to solve diagnostic issues and doubts pertaining to morphological observation.

References

Application of diagnostic classes in thyroid cytology

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Introduction. Fine needle aspiration cytology is traditionally used in the diagnosis of palpable thyroid nodules and, in particular, ultrasound-guided FNAC guarantees high levels of sensitivity and specificity, making it possible to examine non-palpable lesions that are even smaller than 0.5 cm in diameter. The use of diagnostic categories shared by clinicians represents an indispensable element in an integrated medical approach in selecting patients for surgery or follow-up.

Methods. Since 2002, we have been applying the diagnostic classes to all ultrasound-guided thyroid fine needle aspirates, and retrospectively we also applied the same classes to all needle aspirates in the previous 5 years, from 1998 to 2001, on the basis of the SNOmed codes, reconverted to diagnostic classes. Thus the present study spans a 10 year period (1998 to 2007) involving 18,375 examinations.

Our classification is somewhat similar to other systems described in literature \(^1\), but is most similar to the classification recently proposed by SIAPEC \(^4\).

This system utilizes the five classes currently in use in other fields of diagnostic cytology, where there are two sub-categories between malignancy (C5) and benignancy (C2) – one defined as “indeterminate” (C3), closer to benignity, and the other defined as “suspicious” (C4), closer to malignancy. We have classified follicular neoplasms in the “indeterminate” category, together with cases that showed nuclear alterations indeterminate and insufficient for suspected papillary carcinoma, and the atypical lymphocyte proliferations without epithelium, which place the differential diagnosis between thyroiditis and lymphoma. We then defined the “inadequate” category as C1, and for each category, we tried to find precise reproducible morphological criteria.

Results. A total of 18,375 cytological samples from 15,261 patients were analyzed, with the following results.

C1 (“inadequate”). The mean of the inadequate samples decreased from 20.7% in 1998 to 9.7% in 2007. If we consider that, unlike other case series \(^6\), there is no immediate evaluation of the adequacy of the material, consistent with what has been reported in a large literature review by Gharib et al. \(^4\), which showed a percentage of inadequate samples between 2% and 21%, with an average of 17%, for the more than 18,000 cases considered.

C2 (“benign”). The result of 75.6% falls within the mean values, with little variability over the years. In the review by Gharib et al. \(^4\), the percentage of benign cytological samples ranges from 53% to 90%, with a mean of 69%; in the other large cohorts \(^5\), the percentage ranges from 37% to 87%.

C3 (“indeterminate”). The percentage reported by us in this category, which we can define as the actual “grey area” of thyroid cytology, is 8.2% compared to 10% cited by Gharib et al. \(^4\) and 18.6% as reported by Poller et al. \(^4\). This result is satisfactory if we consider that this category also includes the “follicular neoplasms”, included by many in the category of “suspicious”, because of the objective impossibility of characterizing them by cytological examination alone. The follicular neoplasms, varying in percentage from 10% to 20% \(^10\), mostly consist (as seen from histological reports) of follicular adenomas, insofar as they also include follicular carcinomas and follicular variants of papillary carcinomas. The risk of surgical “overtreatment” which seems to be inevitable in case of “suspected” cytological diagnosis, and which can involve up to 85% of patients \(^14\), makes stringent application of cytological criteria for differentiation between “indeterminate” and “suspicious” fundamental.

C4 (“suspicious”) and C5 (“malignant”). These two cytological classes do not exceed 5% and for which the diagnoses of malignancy always exceed the diagnosis of “suspicious”. Since this evaluation is followed by surgery, it is possible to confirm the data or evaluate the reasons for false positives.
Cytological-histological correlation was possible for 1576 patients, i.e. 10.3% of 15,261 patients who underwent thyroid fine needle aspiration.

1. Of the 2267 needle aspirates with diagnosis of C1 (inadequate) corresponding to 1754 patients, 91 patients underwent surgery. Of these, in 65 patients (71.4%) the diagnosis was benign lesion, while 26 (28.5%) were diagnosed with a malignant tumour. Malignant tumours therefore constitute 1.4% of the cases initially classified as C1, a value definitely lower than that previously reported.

2. Of the 1501 needle aspirates with diagnosis of C3 (indeterminate) corresponding to 1152 patients, 382 patients underwent surgery. Of these, 245 patients (64%) were found to have benign lesions, while the lesion was found to be malignant in 137 cases (35%). In the group where all the lesions were cytologically classified as C3, the malignant lesions constituted 11.8%.

3. The 11 false positives were all cytologically classified as C4 and histological examination showed the following:
   - 4 (36%) Hurthle cell adenomas;
   - 4 (36%) adenomatous hyperplasia;
   - 3 (27%) follicular adenomas.

4. The 57 false negatives were histologically found to be:
   - 27 (47.3%) papillary microcarcinomas (average dimensions of 0.5 cm);
   - 11 (19.2%) follicular variant of papillary carcinomas;
   - 7 (12.2%) classic variant of papillary carcinomas;
   - 7 (12.2%) follicular carcinomas (5 minimally invasive and 2 widely invasive);
   - 3 (5.2%) medullary carcinoma;
   - 1 (1.7%) diffuse sclerosing carcinoma;
   - 1 (1.7%) renal clear cell carcinoma metastases.

While the 27 papillary microcarcinomas not diagnosed were probably due to lack of sampling, the remaining 30 cases constitute interpretation errors.

The sensitivity was 90%, the specificity was 97%, and the overall accuracy was 98%.

The positive predictive value of C5 is 100%, while that of C4 is 93%.

Conclusions. The application of diagnostic classes in thyroid cytology is an integral part of a therapeutic diagnostic approach shared with endocrinologists, surgeons and nuclear physicians, i.e. all those involved in the treatment of the “thyroid nodule”, and provides excellent results in terms of sensitivity, specificity and diagnostic accuracy. Precise and reproducible morphological criteria must correspond to the diagnostic classes. Ultrasound-guided fine needle aspiration cytology and the pathologist, are therefore essential in a diagnostic therapeutic approach to carcinoma of the thyroid.

References
9. Leenhardt L, Grosclaude P, Chéré-Challine L; Thyroid Cancer Committee. Increased incidence of thyroid carcinoma in France: a true epidemic or thyroid nodule management effects? Report from the French Thyroid Cancer Committee- Thyroid, 1004:14:1056-60.
12. Yang GCH, Liebeskind D, Messina AV. Should cytopathologists stop reporting follicular neoplasms on fine-needle aspiration of the thyroid? A report of the follicular neoplasms on fine-needle aspiration of the thyroid? Diagnosis and histologic follow-up of 147 cases. Cancer (Cancer Cytopathol) 2003;99:69-74.

The old Pap smear: any future?
C. Gentili

The battle against cervical cancer began many years ago. The pioneers were George Papanicolaou (1883-1962) and Hans Hinselmann (1884-1959). Although they lived at the same time, they never met each other and neither saw the exceptional results of their discoveries.

In fact, colposcopy, with few modifications, is still the basic method for the management of cervical lesions, and organized cervical screenings are essentially based on exfoliative cytology.

At the beginning, Papanicolaou’s proposed method for detecting cervical cancer with cytological analysis was met with some difficulty. His first paper, “A new cancer diagnosis” published in the Third Race Betterment Conference, held in Battle Creek Michigan in 1928, was neglected by gynaecologists who preferred to use traditional methods of diagnosis such as biopsy and curettage. Likewise, pathologists were against cytological diagnosis and so Papanicolaou, disappointed, focused on hormonal cytology. After some years, he returned to study the detection of cancer using cytology.

In 1939, in collaboration with the gynaecologist Herbert F. Traut, he confirmed that cytology could diagnose precanceros changes of cervical cells. In 1941 they published their paper in the American Journal of Obstetrics and Gynaecology entitled “The diagnostic value of vaginal smears in carcinoma of the uterus”, where they emphasized the need to develop a simple, inexpensive method of diagnosis that could be applied to large numbers of women in the cancer-bearing period of life”.

They described in detail the technique for collecting cellular debris, smearing it upon glass slides and staining such that the various components may be studied. He also described the findings indicative of carcinoma of the cervix and those that are suggestive of carcinoma of the vagina or vulva.

This new method was accepted favorably, and could be readily applied as a preventive measure. In the monograph ‘Diagnosis of uterine cancer by vaginal smears’ by Papanicolaou, the Pap smear was established in the Third Race Betterment Conference as a simple, inexpensive, effective, and inexpensive method of diagnosis that could be applied to large numbers of women in the cancer-bearing period of life.”
colau and Traut (December 1943)\(^3\), the contents of which had already been revealed several months before in a short article\(^4\), the authors documented the differences between normal and pathologic findings that included nearly 200 cases of cervical cancer.

The first series of cytology courses were given in Cornell University in 1947 and attended by hundreds of physicians worldwide.

By 1948, a cytology programme for the prevention of cancer started with the assistance of the American Cancer Society. By the beginning of the 1950s, the validity of the Pap test was definitively established and the international community crowned the efforts of the Greek researcher. In 1960, the American Medical Association began to recommend annual pap smears. Dr Papanicolaou died only two years later, before the efficacy of pap smears was widely known and before his test was widely used.

Organized screening began in many countries in the 1970s, which marked the turning point in the decline of the disease. Today in Europe more than 30 countries perform screening programmes for cervical cancer, and every year about 114,000,000 women undergo the test.

The ‘Pap’ smear, named as such in honor of Papanicolaou, has saved the lives of millions of women. The mortality rate from carcinoma of the cervix has decreased from 14 per 100,000 women in the 1940s to 4 per 100,000 in 1989 and less than 3 per 100,000 in the 2000s.

However, in recent years, the sensitivity, specificity and the reproducibility of this test has been the object of controversy, raising doubts about the centrality of cervical cytology in screening programs.

**The classifications.** Papanicolaou understood that, as there were different findings between negative and positive smears, a terminology was needed that took into consideration a large group of questionable situations. Such a system should be easy to understand and tailored for clinical aims. In 1954, in the Atlas of exfoliative cytology\(^5\) he introduced the following classifications:

- Smears cannot always be judged as positive or negative. There are cases in which cytologic findings are inconclusive. A classification taking into consideration the relatively large group of questionable smear findings is therefore necessary. One may often experience great difficulty in classifying cells that deviate from the normal type, but show no malignant characteristics. An intermediate class between entirely normal and suspect groups thus appears to be necessary. A similar need for subdivision exists in the positive group. There are instances in which the results are of an overwhelmingly positive character, leaving no doubt as to their final interpretation. On the other hand, there are cases in which there is strong, but not fully convincing, evidence of malignancy. These considerations led us to the acceptance of the following system of classification for cytologic findings, consisting of five groups:
  - Class I – Absence of atypical or abnormal cells.
  - Class II – Atypical cytology, but no evidence of malignancy.
  - Class III – Cytology suggestive of, but not conclusive for, malignancy.
  - Class IV – Cytology strongly suggestive of malignancy.
  - Class V – Cytology conclusive for malignancy.

This classification was successful and has lasted for a long time: in some countries is still being used today. In an attempt to correlate cytology with histology on corresponding tissue specimens, the terms dysplasia and CIS were also introduced in cytology by Reagan (1953)\(^6\) replacing the term of dyskaryosis by Papanicolaou. These concepts and terms were re-evaluated by the World Health Organization in Riotton’s book “Cytology of the female genital tract” (1973)\(^7\). This classification system, designed primarily for histopathology, was well accepted and used in many laboratories in the U.S. and Europe.

However, the system does have some its limits. It appeared clear that even if the various grades of dysplasia were described and classified rigorously, it was very difficult to achieve a consensus with respect to the morphologic criteria necessary for diagnosis and correlation between cytology and histology\(^8\)-\(^10\).

Richart (1973)\(^11\) introduced the concept that lesions of the uterine cervix represent a continuum of disease stages that progress toward invasive cancer. This not only simplified the terminology but had the merit of setting a clear limit between preneoplastic lesions (CIN) and carcinoma. In Richart’s system, CIN1 corresponds to mild dysplasia, CIN 2 to moderate dysplasia and CIN 3 to severe dysplasia and carcinoma *in situ*.

Nonetheless, this classification, conceived both for cytology and histology, did not resolve the problems of previous classification systems not only regarding the intra- and inter-observer reproducibility and the correlations between cytology and histology, but also for intermediate grades\(^12\)-\(^14\). It also rendered difficult the possibility to distinguish CIN 1 from HPV-related flat condylomatous and reactive changes\(^15\). Years later it was proposed to designate those doubtful cases as “borderline CIN”\(^16\) or CIN with HPV-related changes\(^17\).

Until 1988, all three of classifications, plus many modifications, were in use worldwide, resulting in confusion among clinicians, pathologists and researchers.

The advent of the Bethesda System\(^18\) was designed to bring uniformity into the reporting of cervical Pap smears and to implement a system that was congruent with the current understanding of neoplastic process. It also represented an attempt to decrease inter-observer variability by decreasing the number of diagnostic categories.

The advantages of the Bethesda system compared to previous ones are briefly:

- a statement of adequacy of the sample;
- a descriptive diagnosis divided in several general categories (infective, reactive, epithelial abnormalities, glandular abnormalities, etc.);
- only two categories of epithelial abnormalities: LSIL encompassing HPV, mild dysplasia and CIN1 and HSIL encompassing moderate dysplasia, severe dysplasia, CIN 2, CIN 3 and CIS;
- the introduction of the ASCUS/AGUS category.

This latter category has had many critics\(^19\), but, in my opinion, has the merit of having settled the group of questionable findings described by Papanicolaou as “cells that deviate from the normal type but show no malignant characteristics”. Those same critics describe today these abnormalities using an even more obscure and vague terminology. A revision of Bethesda was made two years later\(^20\), and the last revision was made in 2001\(^21\).

The 2001 workshop was very successful. A few important modifications were made, the most significant of which were:

- new criteria about the evaluation of sample adequacy;
- elimination of the “reactive changes category”;
- a better definition of the ASCUS/AGUS category (ASCUS was divided in 2 subcategories: ASC-US ASC-H, and AGC in 5 subcategories.)

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\(^1\) Papanicolaou and Traut (December 1943)

\(^2\) Papanicolaou and Traut (December 1943)

\(^3\) Papanicolaou and Traut (December 1943)

\(^4\) Papanicolaou and Traut (December 1943)

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\(^19\) Papanicolaou and Traut (December 1943)

\(^20\) Papanicolaou and Traut (December 1943)

\(^21\) Papanicolaou and Traut (December 1943)
The Bethesda revision was a good interface for communication between cytopathologists and clinicians, and also for this reason is now the most widely used classification system. Even if for over 60 years the Pap test is still used as a screening method, it has many weak points. In a famous editorial in 1989, Koss defined the Pap test for cervical detention both a triumph and a tragedy: the triumph was the remarkable contribution to the prevention of cervical cancer, while the tragedy was the frequent failure to detect neoplastic diseases.

He identified the following points of failure, many of which are due to the cytology laboratory: 1) inadequate samples; 2) insufficient time devoted to screening; 3) human fatigue; 4) inadequate clinical component; 5) inadequate patient compliance; 6) poor reproducibility of diagnoses; and 7) ineffective aftercare.

The scientific literature is full of studies about the reproducibility, sensitivity and specificity of the test. The major conclusion is that the reproducibility of the test is limited, as stated by IAC Task Force (Schenck et al., 1998) 23. The sensitivity varies from 6 to 55% in Shingleton’s and Lieu’s papers (1996) 24,25, and was confirmed by several successive studies comparing sensitivity between cytology and HPV test (for an updated review see Koliopoulos et al., 2006 26 and Cuzick et al., 2008 27). Commenting on the study called by the Canadian Cervical Cancer Screening Trial (C3CaST) 28, Eduardo Franco told CTV News (2007): “The Pap test was actually only a bit better than a coin flip to detect pre-cancerous lesions, just 55% compared with the HPV test, which has a sensitivity of 95%” (sic!). Moreover, to maintain clinical performance, laboratories need efficient programmes of quality control as well as expert staff that undergo regular retraining. Despite the low cost of the basic equipment and reagents, high quality cytology is expensive in absolute terms and may not necessarily be the most cost-effective option for screening 27.

New technologies in cervical cytology. Liquid-based cytology. At the beginning of the 1990s, researchers began looking for ways to improve the quality of the Pap test and began to use new systems to collect, preserve and examine samples 29,30.

The new systems consisted in putting the mucus taken from the cervix in liquid fluid containing a fixative and lysing agents instead of putting it directly onto the slide. The sample, processed by filtration or centrifugation, produced results with a clean background, no clumping or overlapping of cells, an even distribution of cells and a good preservation properties. This promising new method was approved by the FDA in 1997.

The supporters this new Pap smear maintained that it provided a improvements in test performances (sensitivity, reproducibility, adequacy, etc.). However, others claimed that the results were substantially the same, except with a much higher cost.

At present, despite extensive study, the debate is still ongoing 31,39.

These studies can be summarized in 9 points:
- LBC system;
- reduces the average time for screening;
- reduces the number of inadequate slides;
- detects an increased number of LSIL with statistical significance;
- detects an increase of HSIL with statistical significance;
- is a platform for saving material for performing other tests;
- is more suitable for automated cytology; but
- the higher number of low grade cellular abnormalities requires additional tests to detect the presence of a histological high grade CIN (i.e. HPV triage testing);
- is still a morphologic technique with the same limitations as conventional cytology; and, above all,
- is more expensive than the traditional test.

Despite the controversies over this new test, it is gaining popularity.

In the U.S., at least 80% percent of all Pap tests are LBC; LBC is also widely used in Europe: it is the standard in England, it is used in > 60% Pap tests in Switzerland, > 70% Pap tests in Belgium, and is the only screening test used in Scotland and Ireland; LBC is approved for routine screening in Hong Kong and Canada; IARC/WHO (2005) approved LBC as an “effective method of cervical cancer control”: European Guidelines for Cervical cancer Screening approved LBC (2007) 40.

Computer-assisted cytology

Many attempts to automate the screening process have been made since the 1950s. The challenge was to reproduce the work of cytotecnologists and pathologists using electronic images and a computer. The first goal was to fully automate the process which is made difficult by the complexity of interpreting the Pap smear, which was previously done by highly trained staff. Two systems, the PAPnetR system and the AutoPap, were approved by the FDA in the 1990s just to screen (for quality control) previous manually screened conventional negative Pap-smears. The AutoPap system, now called FocalPointTM, was approved for primary screening in 2001 as was another system, the ThinPrepR Imaging System in 2003. The PAPnet system had no success, and several factors led to its failure 41. The system required viewing the most representative image of digitized slides on a monitor, which was a difficult adjustment for many people. The slides were sent to a remote centre to be scanned, and the digitized images were sent back for review making it a very complicated method. The costs were high and the system, based on review of conventional Pap smears, did not demonstrate a significant increase in disease detection 42.

The FocalPointTM Slide Profiler is designed so that all of the slides (traditional and LBC) are processed. It allowed about 25% of slides to be immediately archived with no further review, and the remainder are screened manually.

The Thin PrepR Imaging System scans and locates, on Thin-Prep slides, 22 fields of clinical interest.

The cytotecnologist reviews each slide at selectareas for further review by a pathologist, and archives the negative cases 43.

There are a number of reasons to develop automated screening systems for Pap tests. One of the most important is that the evaluation of Pap tests is an intensive process, and the number of Pap-smears that can be screened by a cytotecnologist in a given period of time is limited by regulatory restrictions. Moreover, many recent publications support the efficacy of automated screening in terms of increased detection of LSIL-HSIL, and reducing unsatisfactory results 42-46. However, although these new technologies have improved the overall sensitivity of cytological performance, the costs of the equipment preclude its application in all laboratories.

Immunochemistry. The P16 protein and HPV L1 capsid protein tests are reproducible and specific, and provide indirect information about the status of the integration of these viruses in cellular DNA. They have a prognostic value in ASCUS/LSIL in predicting the progression of the disease and producing an estimation of its carcinogenetic potentiality 47-48.
Molecular biology tests. In recent years, molecular biology has made possible several tests for diverse clinical uses that may change the strategy for cervical cancer control. There are now tests for HPV DNA that detect the presence of the high risk virus, genotyping that discriminates HPV16 from other high risk types, and tests for mRNA that detect messenger RNA from the E6 and E7 genes of high risk viral types. Tests on biologic liquids are being used and recommended by international guidelines and scientific bodies, and have improved the management of epithelial abnormalities such as triage of ASCUS, triage of LSIL, subsequent management after negative findings on colposcopy and follow-up of CIN lesions.

Several recent studies have promoted molecular screening as a reasonable alternative to cytology. Cuzick, in a review and update of recently published meta-analysis and systematic reviews on the HPV DNA test as primary screening compared to Pap smears in the primary screening (more than 30 studies were taken into consideration), shows clearly that:

- a gain in sensitivity close to 30%, and a loss of specificity around 4%-6%;
- better longitudinal sensitivity at 10 years;
- higher reproducibility and automation;
- high throughput.

- a reduction of costs possible from validated competition in molecular technologies, and more screening programs adopting an HPV DNA test (difficult in cytology where the quality depends mainly on human resources).

Cuzick summarizes in 4 points the advantages of using HPV testing as the sole primary screening modality:

- provides an automatic, objective and very sensitive test (better quality control, reduction of medicato-legal claims);
- reserves cytology for HPV positive women (5%-15%) (high quality in cytology, fewer and more focused cyto-screeners);
- avoids the unnecessary triage of HPV negative ASC-US/LSIL;
- permits a longer screening interval (improvements in cost and convenience of screening).

Future prospects for cervical cancer screening and conventional Pap smear. In summary, Pap smear is a highly specific low sensitive exam for detecting cervical cancer, although this doesn’t make the Pap-smear the most effective tool for screening. However, until a few years ago there were no other suitable diagnostic tests, but at least Pap smears were economical, non-invasive, had sufficient reproducibility and thus a good overall choice. Today the role of Pap-smears is being reconsidered in the light of two new frontiers in the field of HPV diseases. First of all, as already mentioned, several new techniques can be used that appear to be more feasible for cervical cancer screening, even if current screening programs still uses exfoliative cytology. As a matter of fact, testing only HPV DNA positive patients has the potential to reduce Pap-cytology by 85%-90%. Moreover, the use of HPV typing for 16 and 18/45, P16 and for mRNA coding for the viral E6/E7 proteins, would further improve the low specificity of HPV testing due to transient infections. Thus, HPV typing has the potential to even further reduce the role of Pap cytology. HPV testing and genotyping can become a reality in routine diagnostic cytology when its specificity and reliability are satisfactorily increased. We claim that reliability can further increase either because medical staff will focus on selected cases or because new prognostic indications will enter into clinical practice, improving reproducibility and giving clinicians the indications for management of positive cases.

The urgency to develop a more sensitive test will become even stronger when we are faced with the changes of HPV epidemiology following the arrival of the HPV vaccine era. In fact, assuming that vaccination leads to a significant reduction in HSIL and relatively more cytological abnormalities due to reactive changes or LSIL, a much more sensitive test will be required to detect the rare cases of cancer precursors and combine it with another test which has a high degree of specificity.

References

29. Shingleton HM, Patrick RL, Johnston WW, Smith RA. The current
in primary cervical screening: a systematic review and meta-analysis
options for cervical cancer screening in developed and developing
33. Mayrand MH, Duarte-Franco E, Denault T, Berger B, Cibas EAS. A new
look at cervical cytology. ThinPrep multicenter trial results. Acta
34. Austin RM, Ramzy I. Increased detection of epithelial cell abnormalities
by liquid-based gynecologic cytology preparations. A review of
35. Bernstein SJ, Sanchez-Ramos L, Ndubisi B. Liquid-based cervical
cytologic smear study and conventional Papanicolaou smear: a
meta-analysis of prospective studies comparing cytologic diagnosis
36. Abulafia O, Pezzullo JC, Sherrer DM. Performance of ThinPrep
liquid-based cervical cytology in comparison with conventionally
prepared Papanicolaou smears: a quantitative survey. Gynecol Oncol
2003;90:137-44.
37. Colgan TJ, McLachlin CM, Cotterchio M, Howlett R, Seidenfeld AM,
Mai VM. Results of the implementation of liquid-based cytology-Sure
A. Effect of study design and quality on unsatisfactory rates, cytology
classifications, and accuracy in liquid-based versus conventional cycler
C. Accuracy of liquid based versus conventional cytology: overall
results of new technologies for cervical cancer screening: randomised
40. Strander B, Andersson-Elström A, Milsom I, Rådberg T, Ryd W.
Liquid-based cytology versus conventional Papanicolaou smear in
an organized screening program: a prospective randomized study.
41. Arbyn M, Bergeron C, Klinkhamer P, Martin-Hirsch P, Siebers AG,
Bulten J. Liquid compared with conventional cervical cytology: a sys-
(eads). European guidelines for quality assurance in cervical cancer
43. Lozano R. Comparison of computer-assisted and manual screening of
44. O’Leary TJ, Tellado M, Buckner SB, Ali IS, Stevens A, Ollayos
CW. PAPNET-assisted rescreening of cervical smears: cost and ac-
ccuracy compared with a 100% manual rescreening strategy. JAMA
manual screening in the detection of squamous intraepithelial lesions
46. Lozano R. Comparison of computer-assisted and manual screening of
47. Miller FS, Nagel LE, Kenny-Moynihan MB. Implementation of the
ThinPrep imaging system in a high-volume metropolitan laboratory.
48. Davey E, d’Assuncao J, Irwig L, Macaskill P, Chan SF, Richards A,
et al. Accuracy of reading liquid based cytology slides using the Thin-
Prep Imager compared with conventional cytology: prospective study.
49. Klaes R, Benner A, Friedrich T, Ridder R, Herrington S, Jenkins D,
et al. p16INK4a immunohistochemistry improves interobserver agree-
ment in the diagnosis of cervical intraepithelial neoplasia. Am J Surg
50. Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, Gillio-Tos A,
De Marco L, et al.; NTCC Working Group. Use of p16-INK4A over-
expression to increase the specificity of human papillomavirus testing:
a nested subset of the NTCC randomised controlled trial. Lancet
51. Rauber D, Melnhom F, Fasching PA, Beckmann MW, Ackermann S.
Prognostic significance of the detection of human papilloma virus L1
protein in smears of mild to moderate cervical intraepithelial lesions.

Educational roundtable in cytology: legal
value of a Master’s degree

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‘Roma; ‘Torino; ‘Milano; ‘Napoli; ‘Modena

A roundtable on education in cytopathology was held on December 1, 2008 at the IV Symposium of Cytology in Bologna. Professor R. Navone initiated the discussion with a historical review of the teaching of cytology in Italy. From 1970 to 1990, different institutions throughout the country offered specific courses – lasting from 6 months to 2 years – to encourage expertise in cytopathology. The courses were open to students of varied academic backgrounds, (MD, BS in biological sciences, or technical degrees) and were attended by over 2,000 individuals. The need for a “cytologist” as a specific professional figure was advocated by the National Oncological Committee (GU 127, 1/06/1996), and by the State and Regions Conference (GU 102, 2/05/2001). Yet the courses were never recognised legally as professional upgrading.

Cytological training was part of the national curricula of the 3-year Clinical Laboratory Technician degree (“Diploma universitario per tecnico di laboratorio biomedico”) from 1992 to 1998. When University rules changed to comply with the European Sorbonne Protocol, the specific courses in cytology were terminated.

Prof. F. Rivasi stressed the importance of the University Master Courses both as a response to the need for academic courses according to the new European regulations, and for formal, recognised training. Although recognised as academic degrees, the vast number of new MS courses offered by Italian universities have had no impact on a candidate’s professional CV.

The University of Rome I “La Sapienza” and the University of Turin established a first level MS course in “Diagnostic Cytopathology in Screening” in the 2003/2004 and 2004/2005 academic years respectively. These MS courses were designed to address the gap in institutional training, to update cytoscreening, addressed in particular to graduates of the Biological Sciences and Biomedical Laboratory Technicians’ Short Course.

The courses, attended by about 100 students, could only partially fulfill the need for trained cytoscreeners, who are essential to for Italian programs for cervical cancer screening. The round-table participants emphasised the importance of official recognition of this new Master’s degree.

Prof. Bulfamante suggested that courses in cytology, both for cytoscreeners and pathologists, could be offered by the University in accordance with regional health programs, thus
extending professional recognition. Prof. Buffamante also emphasised that training in cytology for pathologists should not be limited to cervical screening, but could be incorporated into the School of Surgical Pathology.

At the discussion that followed the round-table presentation, it was suggested that since specific training was not offered by all postgraduate medical school programs, that a teaching net be established within different universities with the cooperation of associated teaching hospitals having the requisite cytological expertise.

There was a strong request voiced by many participants of different backgrounds to improve cytological teaching at all levels, with courses recognised and supported by the Italian Society of Pathology and Cytology (SIAPEC). Prof. Navone proposed that in order to increase the value of cytological screening as a specific and recognised skill in the medical profession, a National Aptitude Test recognised by SIAPEC – similar to the recognised European EFCS-QUATE Aptitude Test in Cervical Cytology – be administered in Turin, just before the upcoming 2009 Cytological Symposium.

Standardization in urinary cytology

G. Montanari, A. Sciacca

“The Secretary of Health of the Piemonte Region has created an ad hoc Working Group in order to produce methodological guidance for the proper execution of tests of urinary cytology, including criteria for quality control (similar to what has been done for cervical cytology). The Group includes a variety of experts including pathologists, clinicians, and epidemiologists. The aim is to reduce the number of ineffectual requests for a diagnosis accompanied by insufficient (and thus inadequate) material. This particular circumstance brings about additional collection of urine from cancer patients or workers exposed to bladder carcinogens, thus implying increased stress and wasted time, as well as financial loss by the National Health Service. In May 2008, the Group published guidance for methods of urine collection and for the preparation of cytological tests, as well as procedures for quality control. The critical issues have been analysed and suggestions for improvement have been made.

Urinary cytology is a laboratory procedure whose major use is the search for neoplastic cells exfoliated from the epithelium of the bladder and other segments of the urinary tract. At present, 60 years after the original studies of Papanicolaou and Marshall, it is still widely used for diagnosis and monitoring of urothelial cancer. It is non-invasive and well-accepted by patients. Its major flaw is its low sensitivity in the detection of low-grade cancer, while its accuracy depends on the preparation of the material and experience of the operator. Given the low sensitivity of the traditional test, new approaches and methods have been recently proposed, although they have not yet been validated.

At least in Piemonte, the paucity of indications for quality of both preparing the material and reading the test, each institution – regardless of whether in private or public institutions, officially recognized or not – has developed its own procedures according to local criteria based on the availability of resources, staff, and experience in the specific area. This has brought about heterogeneity between laboratories, with obvious consequences on the test’s efficiency.

Thus, the Group addressed its activity as related to the production of uniform and univocal protocols.

Worldwide and in Italy, bladder cancer is respectively the ninth and fourth most frequent cancer. In recent years, around 350,000 cases occur yearly worldwide: men account for three quarters of all cases. The incidence is higher in industrialized countries. Currently, in Italy, the cumulative risk of being diagnosed a bladder cancer before age 74 is 41.6 and 7.2 per 1000, respectively, in men and women 3. In the city of Turin, from 1993–1998 the incidence rates (per year per 100,000) were 39.3 in men and 7.1 in women 4. In Italy, since the late 1980s, mortality from bladder cancer is decreasing, with no major differences among geographical macroareas. In 2000–2002, in Italy, the yearly average number of deaths from bladder cancer was 4067, corresponding to rates of 10.3 and 1.6 per 100,000 in men and women, respectively 4. Details on occupational exposures entailing a high risk of bladder cancer can be found in the Monographs of the International Agency for Research on Cancer. In Italy, Decree 25/2002 on the protection of workers, has addressed the issue of health surveillance.

The sensitivity and specificity of urinary cytology, as well as comparability of the accuracy between observers with different training or expertise have been rarely estimated, although a number of reports aimed at comparing traditional cytology to new tests (BTA, NMP22, FISH) are now available. Given its limited sensitivity, traditional cytological urinary is not adequate for a screening programme. Nevertheless, it is valid tool particularly in symptomatic patients (dysuria, haematuria). Its sensitivity and specificity largely depend on the methods used for collection of the material, and this should be kept in mind when formulating a diagnosis. Similar (and even more obvious) considerations are valid for the standardization of the methods for processing the biological sample. For the time being, criteria for reporting the diagnosis of a urinary cytology test have not been standardized: those indicated by the Papanicolaou Society for cytology are recommended. The reporting system should be linked to the protocols of the second level (dagnostic-therapeutic). The cytological laboratory should process a number of tests adequately to ensure that quality is preserved.

In Piemonte, approximately 100,000 cytological urinary tests are carried out each year, 85% of which are in public institutions. Nevertheless, in 2006, less than one third of public laboratories (15 of 48) have carried out more than 2000 tests. Among 27 private (officially recognized) institutions, the standard has been reached only by one. Thus, there are reasons for suggesting centralization of test evaluation in laboratories reaching at least 1000 samples per year, together with the implementation of more frequent and coordinated quality controls. Finally, in the real world, quality is the result of proper basic training and experience supported by a large series of cases and continuous education. The Region, in agreement with the Italian Society of Pathology and Cytology (Società Italiana Anatomia Patologica e Citologia – SIAPEC – Sezione Piemontese) should implement appropriate programmes and verify the quality of the operators in urinary cytology, through biennial evaluations of sensitivity and specificity. Comparisons between laboratories, between public and private laboratories and between laboratories and average regional indicators of quality will be needed.

Finally, estimating the prevalence of cancer using the urinary tract is important (this can be done through the Cancer Registry and hospital admission records). On an individual basis, this information should be linked to the registry of urinary Pap
Liquid-based cytology: practical diagnostic applications in a basic laboratory

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Liquid-based cytology (LBC) has gained popularity in cervical cytology mainly to overcome some limits of the conventional pap smear. The technique (ThinPrep, Cytyc Corporation, Marlborough, MA) was adopted in our laboratory in 1999 for cervical cytology and extended to all non-gynecological samples in 2000, and in particular to fine-needle-aspiration cytology (FNAC). The purpose was to reduce the high percentage of unsatisfactory cases sometimes exceeding 30%-40% of cases especially in thyroid, breast and biliary tract cytology. Such an inadmissible rate was chiefly due to unskilled collection of samples from different outpatient departments, and thus the test could clearly benefit from an easier procedure that requires less technical skills (i.e. flushing cells into a transport medium) in order to obtain a good quality slide.

We have successfully applied the latter method to fluids, body cavity effusions, biliary tract brushing, respiratory tract cytology, FNAC and endometrial sampling.

As far as urinary cytology is concerned, three-day urine collections are concentrated in a single representative vial, thus reducing the number of slides to examine and shortening the reading time.

The growing experience obtained during these years has significantly improved the quality of results and the accuracy of diagnostic performance. In fact, the percentage of unsatisfactory cases is now less than 10%.

Among the most relevant advantages of LBC the following can be mentioned: the smaller the area to examine, the more cells can be uniformly distributed and better preserved, multi-layering and thick areas can be reduced, the contribution of confounding factors such as blood, mucus and air-drying artefacts can be minimized, and slides can be more standardized. In FNAC, however, hypocellular samples can be retrieved by concentrating in a small area even the few cells collected thus reducing the need to recall the patient and to repeat the sampling. Others improvements in diagnostic performance are the possibility to arrange additional slides and to have residual vials available for additional techniques such as immunocytochemistry, molecular biology and viral testing.

Small fragments of tissue, collected from the needle or the sampling procedures, can be lastly sorted out from vials as useful micro-histological cores which are sometimes comparable to true biopsies.

As far as the changeover is concerned, it must be pointed out that a significant training time and practice are needed in order to obtain a progressive confidence with the method, constantly comparing the classical criteria of cytology with the new ones encountered in the liquid phase. Morphological features are, in fact, undoubtedly rather different in non-gyn LBC due to direct immersion of cells in a fixative liquid instead of smearing and fixing them on a slide. Cells are usually smaller and rounder with some loss of spindling, assuming a typical monochromatic shedding of blue and gray: but nuclear details remain unchanged and clearly evident. In any case, classical criteria of conventional cytology are, in our opinion, almost completely preserved: cellularity, morphology, cohesiveness and loss of cohesiveness, background, stromal component and tumour diathesis are evident in ThinPrep slides. Papillae, nuclear grooves, and nuclear inclusions are, for instance, easily recognized in papillary carcinoma of thyroid.

Lastly, it should be mentioned that LBC, especially if supported by sophisticated technical equipment, is more expensive than conventional cytology. However, it is also well known that, under the above-mentioned conditions, the benefits for the laboratory are clear, especially in terms of more satisfactory results, a decrease in repeat procedures, decreased screening time, and a better utilization of human and technical resources.

References


Borderline cytology and predictive morphological aspects of high grade lesions

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Among cytological lesions of the uterine cervix diagnosed as low grade squamous intraepithelial lesions (LSIL) and as borderline categories (ASC US and ASC H) according to the Bethesda 2001 System, it is not rare to find CIN II + histological lesions. For the cytopathologist, this event is not uncommon, since the diagnostic classification suffers from a variability of criteria that are often not known to the clinician and sometimes misinterpreted. Cytological diagnosis is mainly influenced by the adequacy of the sample. In fact, a poorly taken sample, although allowing a diagnosis that is not accurate, makes it difficult to interpret the morphological pattern:
the mucus and the granulocytes that cover the cells, the elongation of the cell structure due to stretching on the slide for preparing the sample, the presence of abundant exudates and blood, etc. Starting from the idea that a Pap smear is adequate when atypical cells are identified, its diagnostic definition is, for example, influenced by the poor representativeness of severe lesion cells with obvious prevalence of a low grade SIL lesion pattern which may or may not be associated with HPV viral cell modifications. In this context, the cytopathologist tends to favour a “prudent” diagnosis such as LSIL, and not HSIL, especially if the evaluation of the slide is clearly limited by the quality of the sample or by inflammation. If the therapeutic approach of the reference gynaecologist is particularly aggressive, with a morphological pattern as described above, the diagnosis in this case is also LSIL, since colposcopic examination is considered to be priority even more than the diagnostic accuracy. Cytological diagnostic underestimation can, however, lead to poor clinical management, especially in case of diagnosis of ASC US and LSIL when the disease is not immediately found during the first colposcopic examination. Moreover, the clinician may be less careful when checking the endocervix for lesions than during diagnosis of HSIL.

In TBS 2001, there is no precise diagnostic approach when significant modifications of ASC H are present on an unequivocal LSIL background. The pathologist’s diagnostic decision is to classify this type of lesion as LSIL or HSIL, and the choice depends on the context. Some studies have proposed another type of diagnosis: LSIL H = LSIL cannot exclude HSIL. A study by Elsheikh shows that in 40% of samples a diagnosis of LSIL H implied histological diagnosis of CIN II + and the percentage was equivalent to the diagnosis of ASC H (44.6%). According to Louro, there is no significant difference in percentage in various studies using a traditional or liquid phase Pap smear. According to several authors, a diagnosis of LSIL H is an intermediate risk for severe histological lesions, the percentage of which is equal to that of ASC H, but intermediate between ASC US-LSIL and diagnosis of HSIL. There has been a proposal to consider LSIL H as an entity separate from LSIL and HSIL, but with higher predictivity for CIN II +.

The distribution of the risk of high grade CIN according to the TBS 2001 categories is shown below, and the data in literature also include the cytological category LSIL H. A study by Owens shows that the prevalence of HPV HR was significantly larger in patients with LSIL H compared to ASC H.

Morphologically, cases of LSIL H where the corresponding histological examination showed a CIN II- CIN III, the unequivocal cytological pattern identified was LSIL associated with ASC H small cells. Shidham describes the presence of small parakeratotic cells with distinct shape with angulated cell borders and cytoplasmic eosinophilia. The cells that were identified as ASC H were atypical cells which seemed to be metaplastic. It must be remembered that, unlike ASC US in which the nuclei are 2.5-3 times the size of intermediate squamous cells, nuclear size does not help to define ASC H as there is also a variation in its size. In the cases described by Shidham, there was slight irregularity of the nuclear outline with slight hyperchromasia, in any case, the N/C ratio was high; the ASCH cells were described as being arranged singly and isolated.

Conclusions. Is LSIL H a separate entity? This type of cytological lesion is sometimes observed during diagnoses, yet we are unable to classify it as a precise entity according to the classifications currently in use. Although it is too early to inserting another cytological category that would create confusion for the clinician, the possibility of including a comment after diagnosis has been formulated according to the TBS 2001 classification, such as “LSIL with ASC H cells”, which can avoid understimation in colposcopic clinical diagnosis and allow careful follow-up of patients if the diagnosis is performed at a late stage.

For better diagnostic help in the identification of cellularity in this regard, Shidham recommends the use of p16.

References

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<tr>
<th>Cytology</th>
<th>CIN I %</th>
<th>CIN II / III %</th>
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<tbody>
<tr>
<td>ASC H</td>
<td>41%</td>
<td>59%</td>
</tr>
<tr>
<td>LSIL H</td>
<td>65%</td>
<td>100%</td>
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Tab. II. % of CIN and HPV HR compared to cytology.
Urinary cytology: 3 X 1

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Urinary cytology is used as investigative tool for diagnosis of urothelial neoplasms and for follow-up of patients with invasive and pre-invasive urothelial cancer. It is rarely used as a screening method in asymptomatic patients. Usually, cytological specimens are collected for three consecutive days which increases the sensitivity of the method. Unfortunately this means that patients must go to collecting-point for three days, which may be problematic for elderly patient. To date, a urine cytology specimen is prepared using polycarbonate membrane filtration or using a centrifuge sediment.

The possibility of fixation of urine has been suggested while collecting all the three specimens on the third day. In this case, urine is mixed with a fixative solution (ethyl or methyl alcohol). The urine added to fixative can be centrifuged and filtered. The thin prep cytology can also be prepared with optimal technical quality and with a reduction in the time for diagnosis, but there is an increase in the time needed for sample preparation. More recently, FISH has been used for evaluation of chromosomal abnormalities in urothelial cells. This method is highly sensitive but very expensive, and to date may be applied only on a limited set of patients.

Bologna urinary cytology project: 3X1
The patient goes to a referring center which indicates what laboratory the patient should go to for the examination for three consecutive days. After this, the patient must collect the report. This is a five steps procedure, and the waiting list is very long: in some periods of the year it can even be 3 months.

With fixation of urine, the five step procedure can be reduced to three steps: the first is presentation to referring center and then to the laboratory where the patient can give all three samples of urine together. The last step is collecting the report. In the laboratory, the three urine samples can be processed together, which reduces the number of the specimens with concomitant reduction of the waiting list.

References

Legal value of University Master

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The size of the University Master system can be illustrated by comparing the number of courses: in the period 2002/03, there were 712 master activated, while in 2003/04 the number rose to 1170, with an increase of 64.3% over the previous year. There is also a movement of expansion in Italy, in which the North activated 46.3% of the masters, and the Center activated 39.5%. Furthermore, the domination of the socio-economic and legal master (35.5%) has given way to the scientific and technical areas (39.7%).

The introduction of masters highlights the significant increase in life-long learning and training that tends to enhance the work experience and practical training as an element necessary for the completion of the training course. The basic characteristics of the master (title, level, region which the master is done, number and names of universities proponents, discipline, number of hours of lessons, a master’s target, educational qualifications and requirements required to access to the master, in addition to professional profiles and job opportunities provided) are to be added to the organizational and financial aspects of the master. While a degree course has training techniques and specific assessments, a master can be seen as a service. In particular, the objectives of the master tend to recognize specific target skills and knowledge, promotion of quality of the service provided, and customer satisfaction. There is thus a clear need to give the University Master an exact definition in Italian legislation. The architecture of the system of teaching universities and new academic degrees has been defined by Decree No. 509 of 3 November 1999, which helped to identify the general criteria for universities to establish (in full autonomy) the structure of the study course, subsequently, with DM. 270/2004, the Ministry has confirmed what is already listed with DM 509 above.

Indeed, following the European perspective, the reform provides for a system in 3 areas institutionally and functionally separate education-university-high artistic and musical-technical training education (IFTS), which provides degrees (L), specialist degrees (LS), degrees of specialization (DS), doctoral research (DS) and includes the University Master at the first or second level. Indeed, paragraph 8 of art. 3 of this Decree defines the University Master as “a course of scientific and high-standing and recurrence training”.

In Italy, the legal recognition of the master is defined by law n.127 of 15 May 1997 and Decree N. 509 on 3 November 1999, which includes the recognition of this qualification abroad. It gives concrete form to role played by universities and training of the applicant (lifelong learning), which is a basic demand from the workplace. In this way, Universities may also have a role in the post-degree market.

The term “University Master”, which is now in common use, is not to be confused with the many existing courses in the public and private sectors. In these courses, the term master does not identify the degree issued, but the course itself. In Italy, the degree of university master is protected by virtue of its legal value, and is issued only by public institutions or authorized institutions.

At present, is still difficult to establish a uniform regulation on the assessment of the master in public competitions. Paragraph 3 of Article 8 of the Decree of 1999, states in general that the attainment of a master requires the acquisition of at least 60 credits (university credits), in addition to those acquired for graduation and the ability to reuse, in whole or in part, these credits. At this time, the Commission chairing a public competition can decide whether or not the educational qualifications obtained during a University Master can be recognized. The difficulty in assessment is tied to the continuing evolution of this type of training, which only in the academic year 2002/03, developed into an experimental training phase. In fact, an obvious difference still exists in terms of requirements for access to a master, the type of training and of effective evaluation of candidates, which does not allow uniformity and standardization of University Master at a national level.
Thin-layer preparations in thyroid fine-needle cytology

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In fine-needle cytology of the thyroid, liquid-based/thin-layer preparations represent a valid method that are alternative to or in addition to direct smears. The advantages of liquid-based processing can be transferred to fine-needle cytology to get better quality preparations without technical artefacts and obscuring elements, to reduce the screening time (screening of a small circular area in thin layer as opposed to multiple smears per case), to save residual material for additional slides for ancillary tests to support suspicious diagnosis, or confirm the origin of a neoplasia or the sampling site.

Cyto-architectural features. The diagnostic accuracy is strictly dependent on the identification of the modified cytological and architectural features induced by mechanical strains of different nature and by metanol-based liquid fixation, to avoid misinterpretation. The diagnostic criteria are prevalently maintained, but the cyto-morphological features are different. The effect of the method that is at first evident at low magnification is the decrease in cell size. In follicular cells, the nuclei are shrunken and the cytoplasm disrupted. Colloid appears as “tissue-paper-like” material or dense droplets rather than a diffuse layer. In adenomatous lesions, any small fragments of colloid are important to avoid false diagnosis of follicular neoplasia. Papillary carcinoma retains all the well-known cyto-architectural features; characteristic to thin layer is fragmentation of branching sheets, single cells, nuclei slightly smaller and nuclear molding, nuclear pseudo-inclusions that are preserved but less evident, and easy identification of typical multinucleated giant cells. In chronic lymphocytic thyroiditis, lymphocytes are decreased in number and dispersed in the background. Oncocytic neoplasia may show more crowded three-dimensional groups rather than a single cell pattern, the cell and nuclear size tend to be smaller and the nucleoli less distinct, the cytoplasm may appear pale and delicate mimicking a histiocyte. In undifferentiated carcinoma, necrosis is clumped around the malignant cell groups, instead of being dispersed in the background.

Diagnostic accuracy. Conflicting results have been obtained regarding diagnostic accuracy in the literature. We also tested whether the diagnostic accuracy on thin-layer was comparable to that on direct smear. Materials and methods. In a study including 549 cases of ultrasound-guided thyroid fine-needle aspirations, we compared the diagnostic accuracy on Papanicolaou-stained thin-layer slides (Thin Prep, Cytyc Inc., MA, USA) with that of air dried, MGG-stained conventional direct smears. A split-sample technique (the remainder in the needle, after the smear, was rinsed in a vial with CytoLyt solution) was used for thin-layer. Surgical follow-up was available in 105 cases. Results. All the different types of lesions were represented in both methods. To evaluate the diagnostic accuracy of the two methods, we classified the lesions according to a tiered “T- CATEGORY SYSTEM”, as suggested by the Group for “Consensus in cytology for thyroid nodule” SIAPEC-IAP 2007, including five categories: T1, inadequate/ not representative; T2, benign/negative for malignancy; T3, indeterminate (follicular lesions in which cytology cannot formulate a conclusive diagnosis); T4, suspicious for malignancy; T5, malignant. Thin-layer T1 was higher (16%) than smeared T1 (12%), because of poor residual material in the needle due to the split sample. T2 included the same number of cases (365) with both methods. T3, T4 and T5 were reduced in thin layer (20, 41 and 30 cases) with respect to smears (32, 47 and 36 cases). Statistic parameters. The absolute sensitivity and complete sensitivity were better for smears than thin-layer, but both had good values. The specificity for biopsy cases was higher in thin-layer, but complete specificity was similar in both techniques. The predictive positive value (PPV) of T5 was 100%, while the PPV T4 was better for thin-layer, but PPV T3 was better in smears. The negative predictive value of T2 was similar in both preparations. The false negative rate was better in smears. There were no false positives with either method. With regards to the correlation between T-categories in both methods, it was important to note that:
- 66.3% of T1 in thin-layer were also classified as T1 in smears. The remaining 33.7% was spread among the other categories, in particular 4.5% of thin-layer T1 was T3 and T4 in smear and 1.1% of thin-layer T1 was T5 in smear, demonstrating that the decrease of T3, T4 and T5 observed in thin layer was not caused by misinterpretation or technical artefacts but by the poor cellularity of the split-sample;
- 75.6% of thin-layer T4 was also T4 in smears, 22% was T5 and only 2.4% was T3;
- 86.7% of thin-layer T5 was also classified T5 in smears and the remaining 13.3% was T4.

Conclusions. In fine-needle cytology of thyroid specimens, liquid based/thin-layer preparations have good sensitivity, specificity and predictive values compared with conventional smears and previously reported values in the literature. The liquid based/thin-layer preparation represents a valid alternative in situations where a smear is not optimal because it is operator-dependent in each phase, from fixation until the transferring of material onto the slide. The diagnostic criteria are generally maintained, and only the cyto-morphological features are slightly different, making training essential for accurate interpretation. Future perspectives. The availability of residual material may allow the application of biomolecular predictors, making it possible to discriminate among morphologically indeterminate/suspicious lesions and to fine-tune clinical management.

References

Liquid-based cytology in cervical cancer screening: the experience of Area Vasta Romagna

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Introduction. The Pap test is still considered to be adequate for identification of precancerous and early neoplastic lesions of the uterine cervix. The use of a Pap test in screening programs allows for a significant decrease in the incidence and, in particular, mortality. The methods of sampling, collection of the cells and preparation of the slides are key phases in the appropriated use and efficacy of the Pap test. The introduction of liquid-based cytology (LBC) has substantially modified the
The technical and diagnostic quality of the Pap test, and has the following advantages:
- optimization of the specimen preparation phase: it is no longer necessary to prepare and fix the slides in gynecologic ambulatories; the gynecologic/obstetric sample preparation time has thus decreases and the quality of the slides is less operator-dependent;
- optimization of human resources: cytologic diagnosis is easier and faster;
- reduction in the number of inadequate specimens and increase in conclusive diagnoses with fewer additional screening procedures (colposcopies, biopsies, etc.);
- improvement of standardization and comparability of the screening processes among the various operators;
- possibility to perform the HPV-test and other ancillary methods for neoplastic markers on the residual cells, without recalling the patient;
- eventual applicability of computer-assisted system for cytologic diagnosis.

LBC is in widespread use in Northern America, where more than 5 million Pap tests/year are performed (about 45% of all Pap tests worldwide are performed in USA). Trials have been made in France and Scotland, where LBC was adopted in organized screening programs. Since 2004, the UK has introduced LBC.

The biggest drawback of LBC is its high costs, and at least 40,000 Pap tests/year are needed to guarantee a good balance between costs and benefits. This knowledge stimulated the Area Vasta Romagna Project, which involved the AUSL of Forlì, Cesena, Ravenna, Rimini and, in particular, the Screening Centers, Consultories and Departments of Pathology.

In this area, with about 1,000,000 inhabitants, about 60,000 screening Pap tests are performed per year. This extensive workload has allowed for centralization of slide prepara-

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**Tab. I. Total number of study women and unsatisfactory rate.**

<table>
<thead>
<tr>
<th>CPS period</th>
<th>LBC period</th>
<th>LCB-CPS Rate ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no.</td>
<td>125,750</td>
<td>49,425</td>
</tr>
<tr>
<td>UNSAT</td>
<td>2.20</td>
<td>1.35</td>
</tr>
<tr>
<td>rate (%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tab. I (continued):

<table>
<thead>
<tr>
<th>Woman age</th>
<th>Total no.</th>
<th>UNSAT rate (%)</th>
<th>Total no.</th>
<th>UNSAT rate (%)</th>
<th>Rate ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-34</td>
<td>29,392</td>
<td>2.68</td>
<td>8,753</td>
<td>1.96</td>
<td>0.73 (0.63 – 0.85)</td>
</tr>
<tr>
<td>35-44</td>
<td>34,726</td>
<td>2.3</td>
<td>14,144</td>
<td>1.43</td>
<td>0.62 (0.54 – 0.71)</td>
</tr>
<tr>
<td>45-54</td>
<td>29,571</td>
<td>2.18</td>
<td>13,474</td>
<td>1.22</td>
<td>0.56 (0.48 – 0.65)</td>
</tr>
<tr>
<td>55-64</td>
<td>32,061</td>
<td>1.68</td>
<td>13,054</td>
<td>0.97</td>
<td>0.58 (0.48 – 0.69)</td>
</tr>
<tr>
<td>Total</td>
<td>125,750</td>
<td>2.20</td>
<td>49,425</td>
<td>1.35</td>
<td>0.62 (0.57 – 0.67)</td>
</tr>
</tbody>
</table>

* Ratio (with 95% confidence interval) between the observed number of unsatisfactory specimens in the LBC period and that expected based on the unsatisfactory rate in the CPS period. Woman age-specific ratios are standardised by health district. Total ratio is standardised by woman age and health district.

---

**Tab. II. Reporting rates and detection rates in the LBC period.**

<table>
<thead>
<tr>
<th>Woman age</th>
<th>No. of woman with valid results</th>
<th>Reporting rate (%)</th>
<th>Detection rate (per 1,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASCUS</td>
<td>AGUS</td>
<td>LSIL</td>
</tr>
<tr>
<td>25-34</td>
<td>8,696</td>
<td>2.44</td>
<td>0.08</td>
</tr>
<tr>
<td>35-44</td>
<td>14,092</td>
<td>2.62</td>
<td>0.13</td>
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<tr>
<td>45-54</td>
<td>13,441</td>
<td>2.36</td>
<td>0.24</td>
</tr>
<tr>
<td>55-64</td>
<td>13,028</td>
<td>1.16</td>
<td>0.12</td>
</tr>
<tr>
<td>Total</td>
<td>49,257</td>
<td>2.13</td>
<td>0.15</td>
</tr>
</tbody>
</table>

---

**Tab. III. LBC: CPS reporting rates ratio a.**

<table>
<thead>
<tr>
<th>Woman age</th>
<th>ASCUS</th>
<th>AGUS</th>
<th>L-SIL</th>
<th>H-SIL/Ca</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-34</td>
<td>1.13 (0.98-1.29)</td>
<td>0.54 (0.22-1.11)</td>
<td>1.46(1.28-1.67)</td>
<td>1.22 (0.86-1.69)</td>
<td>1.25 (1.14-1.37)</td>
</tr>
<tr>
<td>35-44</td>
<td>1.12 (1.01-1.24)</td>
<td>0.36 (0.22-0.57)</td>
<td>1.06 (0.92-1.23)</td>
<td>0.92 (0.65-1.27)</td>
<td>1.02 (0.94-1.11)</td>
</tr>
<tr>
<td>45-54</td>
<td>0.89 (0.79-0.99)</td>
<td>0.45 (0.30-0.61)</td>
<td>0.85 (0.68-1.04)</td>
<td>0.53 (0.28-0.91)</td>
<td>0.80 (0.73-0.88)</td>
</tr>
<tr>
<td>55-64</td>
<td>0.78 (0.66-0.92)</td>
<td>0.30 (0.17-0.50)</td>
<td>0.59 (0.40-0.85)</td>
<td>0.83 (0.42-1.49)</td>
<td>0.68 (0.59-0.77)</td>
</tr>
<tr>
<td>Total</td>
<td>0.98 (0.92-1.04)</td>
<td>0.39 (0.30-0.49)</td>
<td>1.09 (1.00-1.19)</td>
<td>0.91 (0.74-1.11)</td>
<td>0.95 (0.90-0.99)</td>
</tr>
</tbody>
</table>

* Ratio (with 95% confidence interval) between the observed number of cytology diagnoses reported in the LBC period and that expected based on the reporting rate in the CPS period. Woman age-specific ratios are standardised by health district. Total ratio is standardised by woman age and health district.
tion from different centres in only one Cytology Laboratory (Forlì).
The collection of cytological specimens and Pap test diagnoses are performed in the various districts. This was a 3-year Project (2005-2007).
Such a working procedure represented a unique model in Italy.
Another important innovation was the introduction of a high level of automation regarding preparation of slides, which permitted a lower workload for technicians.

Methods. The unsatisfactory rates, the reporting rates, the total positivity rates, the follow-up compliance rates, the detection rates and the predictive values in the liquid-based cytology (LBC) period were compared with those of the conventional Papanicolaou smears (CPS), using their standardized ratio with the 95% confidence interval (CI). Age-specific (10-year groups) ratios were standardized by the sending laboratory. Because the 2006 WHO did not provide cyto-histologic correlations by age-group, the predictive values were standardized only by the sending laboratory. For all these proportions, the numerator observed (O) in the LCS period was divided by that expected (E). This was calculated by multiplying the laboratory-specific (and woman age-specific, if applicable) rate in the CPS period by the appropriate denominator in the LBC period.

Results. The study population comprised 125,750 women in the CPS period and 49,425 in the LBC period. The respective unsatisfactory rates were 2.20% and 1.35%, with an absolute decrease of 85% and a relative decrease of 38%. The impact of LBC was significant in all age groups (Tab. I).

Table III shows the LBC:CPS comparison of abnormal cytologic reporting rates. In the LBC period, the total rate was 5% lower. An increasing rate was observed only for low-grade squamous intraepithelial lesions (L-SIL), with a decrease of ASC-US/L-SIL ratio (1.67 observed, 1.79 expected). The overall decrease was entirely accounted for by women aged 45 years or older, who showed negative variations for all cytologic diagnostic categories. Conversely, 25-34 year-old women showed a 25% increase in the total rate of positive results, mostly due to the L-SIL category. The reporting rate of AGC decreased for all women, although the change was significant only above the age of 34.

As expected, compliance to colposcopic follow-up of women with positive cytology was lower in the LBC period (n = 1452, 82.9%) than in the CPS period (n = 4256, 94.9%). The difference was statistically significant (LBC:CPS ratio 0.87; 95% CI, 0.83-0.92) and was similar across all groups of cytologic diagnoses shown in Table III.

Table IV shows the LBC:CPS comparison of histologic detection rates. The detected prevalence of CIN1 increased by an average of 70% in the LBC period. Because of a strong inverse relationship with the age of women, the increase was significant only under the age of 45.
The total rate of CIN2 was not significantly higher by 19%, although a significant increase of 50% was noted among 25-34 year-old women. We did not observe any significant changes for CIN3/carcinoma.
As a consequence, the LBC:CPS ratio for the CIN2 category was 1.07 (not significant); instead, the LBC:CPS ratio for the CIN1 category was 1.38 or poorer.
Table V shows the positive predictive values of LBC diagnoses (upper part) and the LBC:CPS comparison of positive predictive values (bottom down). In the LBC period, there was a generalized increased in the capability of ASC-US...
to predict all lesions from CIN1 to CIN3/carcinoma; AGC diagnoses predicted more often CIN1-2 lesions and less CIN3/ carcinoma categories compared to the CPS period. Similarly, L-SIL diagnoses exhibited a greater predictive value for CIN1 and an opposite behaviour for CIN2+ lesions. The cyto-histologic correlations between H-SIL and CIN3/carcinoma did not vary significantly. As a consequence, the positive predictive value for CIN2+ lesions was about 70% higher for ASC-US diagnoses and 30% lower for L-SIL in the LBC group. On average, as shown in the right column, ASC-US, AGC and L-SIL had a significantly increased positive predictive value for CIN1+ lesions.

Taken together, as shown in the bottom line of Table V, positive cytolgics in the LBC group demonstrated an increased predictive value only for CIN1 lesions. This accounted for the overall increase of 36% in total predictive value of CIN1+ categories. Because the total rate of positive cytolgics was substantially stable (Tab. II), all ratios in the bottom line of Table V reflected closely the LBC:CPS ratios of the detection rates (bottom line of Tab. IV).

**Conclusions.** The European guidelines for cervical cancer screening clearly define the advantages introduced by liquid-based cytology (LBC) 1. In particular, LBC contributes to a decrease of inadequate Pap tests, makes cytologic diagnosis faster and allows the HPV test to be performed with the same cellular specimen obtained.

In our experience, the introduction of LBC in cervical screening cytology contributed to:
- decrease of inadequate diagnoses;
- increase of L-SIL lesions detected in 25-34 year-old women;
- decrease of the cytologic atypia detected in women > 45 years;
- increase of CIN1 lesions detected in women < 45 years;
- increase of CIN2 lesions detected in 25-34 year-old wom-en;
- higher PPV of ASC-US category for CIN1+ lesions;
- higher PPV of L-SIL category for CIN1 lesions, with an opposite behaviour for CIN2+ lesions;
- more appropriate second level diagnostic procedures for ASC-US cyathogy.

Previous experience has demonstrated the usefulness of the introduction of LBC in cervical cancer screening in large areas 1,3. At a minimum, automation in cytology permits the sharing and centralization of technical procedures. The high costs of LBC, compared with those of conventional cytology, still represent an important drawback. Also according to our experience, a good cost/benefit balance is achieved when a minimum of 40,000 pap tests/year are processed. In our area (Romagna), about 60,000 screening pap tests are performed a year. Therefore, Area Vasta Romagna is a good area in which to introduce an organizational model, which provides centralization of Pap tests in only one Cytology Laboratory. The Area Vasta Romagna Project was conceived according to our experience, a good cost/benefit balance is reached when a minimum of 40,000 pap tests/year are processed. In our area (Romagna), about 60,000 screening pap tests are performed a year. Therefore, Area Vasta Romagna is a good area in which to introduce an organizational model, which provides centralization of Pap tests in only one Cytology Laboratory. The Area Vasta Romagna Project was conceived with the goal of evaluating the possibility of centralization of technical preparation of the cytological slides. In particular, according to our model, a large amount of the screening tests coming from the different centres of Area Vasta Romagna were centrally prepared in the Laboratory of Cytology of Forlì. At the end of this 3-year project, we demonstrated that Area Vasta Romagna is a region in which to apply such a model in cervical screening cytology.

**Acknowledgements.** The author thanks all the colleagues who collaborated in the Project and, in particular, the Department of Pathology, the Screening Centres and the Laboratories in Forlì, Cesena, Ravenna, and Rimini. Moreover, the author thanks the management of the AUSL of Forlì, Cesena, Ravenna and Rimini and Region Emilia-Romagna for financial support.

**References**


**Biological Markers in Breast Cytology**

G. Simone, S. Longo*, S. Petroni, V. Rubini, M. Liuuzzi, M. Caponio, T. Addati, M. Asselti

The first paper on neoadjuvant chemotheraphy was published in 990, utilising Fine Needle Cytology (FNC) to detect prognostic factors before treatment 1. Therefore, the use of cytolgic samples in biological characterization for clinical use, has been a longstanding application of cytopathology. However, the starting point remains morphologic diagnosis, even though additional techniques are assuming a more important role. As commented by Schmitt 2, “Molecular technology applied in the pathology diagnostic field is undoubtedly reshaping the practice of cytopathology. Something new has happened: 1) The introduction of Liquid Based Cytology (LBC) in the Cytopathology Laboratory, also for FNC cell samples; 2) Cytologic specimens have been used successfully for genomic and proteomic studies. As reported by Sneige, “Investigational studies offer great potential in prediction of patient outcomes and decreased response to therapy, as well as assessment of the risk of developing breast cancer”. 3. Despite the its great potential, LBC in breast FNA is still not widely used, probably because pathologists are more confident with conventional smears for diagnostic use. However, it should be underlined that, in addition to diagnosis of malignancy, other information is requested by clinicians for therapeutic purposes, and thus breast cytology is not just for diagnostic use. Therefore, we believe that it is useful to present the preliminary results of an ongoing study in our institution, with the aim of comparing prognostic and predictive breast cancer markers in FNC in LBC and the corresponding primary tumor sample.

To date, we have analyzed 50 tumor specimens (47 CDI, 2 CLI and mucinous carcinoma), among the operable tumors, 20 were > 2 cm and 7 showed nodal involvement.

**Results.** In FNCs of operable cancers 25 of 30 cases were ER positive, 20 expressed PgR and 6 showed a MIB- Index higher than 20%. In metastatic patients, of 20 cases were ER positive, 8 expressed PgR and 6 of 5 evaluable cases showed a MIB- Index higher than 20%. HER/B2-Neu as detected by FISH showed amplification in 2 of 9 operable tumors and in 3 of 2 metastatic cancers. The agreement between the two methods as evaluated by a Spearmann correlation test was 0.74, 0.7 and 0.80 for ER, PgR and MIB+, respectively. Disagreement was present in of 50 cases, 3 of 50 and 6 of 44 for ER, PgR and MIB- , respectively.

Expression of P16 in the functional activity of human papillomavirus E7 oncoprotein in high-risk cervical lesions

A. Nocita, I. Putrino, V. Rossano, M.G. Rania, M. Mauro, F. Tallarigo

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

Introduction. Recent molecular biology data on the natural history of carcinomas of the uterine cervix suggests that viral infection critically interferes with mechanisms of cellular growth and DNA repair. In fact, recent studies have shown an interaction among the high-risk type and the regulators of the cellular cycle: family cyclins, cyclin-dependent kinase (CDK) and inhibitors of cyclin-dependent kinase (CDKI) through the activation and inactivation of mechanisms of phosphorylation. Our attention has been focused on the G1 phase of the “restriction point”, because the most important of regulatory events – induction of proliferation and cellular differentiation - occur in the G1 phase of the cell cycle. In normal cells, hypophosphorylated pRB binds to the E2F transcription factor and inhibits it in G0 and early G1. In proliferating cells, phosphorylation of pRB releases E2F and induces genes that mediate entry into the S phase, regulated by the cyclin E/CDK2 complex. P16 is a product of the CDKN2A gene that suppresses the activity of the cyclins CDK-4 and CDK-6, which regulate the G1 checkpoint. Oncoprotein E7 of HR-HPV disrupts the pRB/E2F interaction, releases active E2F and induces pRB degradation through a proteasome-dependent mechanism. Thus, oncoprotein E7 induces cellular proliferation, and leads to accumulation of gene products that are negatively regulated by pRB, such as p16, E2F-1, Cyclin E and Cyclin A.

Aim: The aim of the following study was to study the biological activity of human papillomavirus E7 oncoprotein by analyzing the level of expression of the tumor-specific gene p16 in cells through the epithelial layer according to CIN grade.

Material and Methods: Twenty-two biopsy specimens were investigated. This cases were classified as CIN III (n = 8), CIN II (n = 4), CIN I (n = 7), and condylomas (n = 2). One negative case was used as a control. All cases were positive infection with high-risk human papillomavirus (HR-HPV).

Results and Conclusion: p16ink4a immunoexpression was compared with histologic grade. The CIN III lesion showed diffuse, full-thickness p16 staining of the epithelium. High-risk HPV types were found by PCR: HPV 16, 31, 58. These results highlight the oncogenic activity of E7 oncoprotein, which induces proliferation asthe E7 protein degrades pRB to release E2F, with accumulation of mutations that lead to loss of genetic integrity. In CIN II lesions, which involved HPV genotypes 16, 31, 33, 56, diffuse p16 staining of two thirds of the epithelium was seen. The biological activity of the E7 oncoprotein is associated with the cyclin E/CDK2 complex, which drives progression into S phase.

The results with CIN I are particularly interesting as they involved HPV genotypes 31 and 33. Im CIN I lesions, focal p16 staining was found in the lower third of the epithelium. While in the case of CIN I in which genotypes 16, 18, and 45 were found, the expression of p16 was negative. In this case, HPV 31 and HPV 33 E7 oncoprotein facilitates replication by activating E2F2 transcription through its interaction with HDACs. The binding of HDACs is also important for the maintenance of viral episomes during differentiation. In conclusion, it can be affirmed that p16 is a marker of biological activity of the E7 oncoprotein.
CR1, CR2 and CR3 based upon homology with adenovirus E1A. CR1 is composed of amino acids 1-20, CR2 spans residues 21-39, and CR3 comprises residues 40-98. E7 dimerizes in the CR3 domain via a zinc-finger motif; this motif being essential for proper folding and viability. E6 oncoprotein is a 151 amino acid protein with two zinc finger domains. E6 is one of the primary oncogenes of the virus, causing immortalization of cells, and together with E7 leads to transformation. E6 binds to the product of the tumor suppressor gene p53, and leads to degradation of p53 together with another cellular protein, namely E6 associated protein (E6-AP). Thereafter, the mechanisms of DNA repair and apoptosis are blocked. E7 interacts with members of the retinoblastoma (Rb) gene family, inducing cell cycle progression. The degradation of p53 and pRB therefore an important mechanism for transformation of normal cervical cells into cancer cells.

Automated ThinPrep Imaging System in Pap test primary screening: the experience of a large provincial area

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Since 1999, liquid based cytology has been adopted in one of our provincial laboratories mainly to reduce the high percentage of inadequate tests both in cervical cytology and in fine-needle aspiration specimens. Considering this, it is of interest to determine why the recently started regional screening programs have recall rates that exceed 10%.

The benefits of the thin layer method are mainly represented by a smaller area to examine, smaller artefacts, reduced inflammatory background, increased adequacy and standardization of specimens and residual vials available for HPV-DNA tests.

With the purpose of introducing a more uniform approach to Pap test screening programs in the Vicenza area, in October 2007 four Pathology Units (Arzignano-Valdagno, Bassano, Thiene-Schio and Vicenza) adopted the automated ThinPrep Imaging System (TIS) of Cytyc Corporation (Marlborough, MA). The project grouped together in the cytology laboratory of Thiene Hospital all the technical equipment (two TP3000 processors, one automated staining station, one Imager System Computer, one Review Station microscope), while 3 remote Review Stations where placed in the other centres. All specimens were collected, processed, stained and imaged in the central lab, while an efficient courier system was set-up to return slides, residual vials and CDs to cooperating pathology units for examination and diagnosis.

TIS was chosen because thin layer procedure was already employed in 3 of the 4 labs, the pre-existing technical background was adequate and the methodological approach of a partial examination of all slides was preferred. The advantages of a large central lab with standardized staining programs include cost reduction, time-savings, uniform quality control and more homogeneous laboratory environment. Critical points include correct specimen identification (with an appropriate uniform bar code for patient, number and centre), increased privacy, transports, printed numbers directly on the slide with no time wasted on paper labels, TIS near - stoichiometric staining and a new way of examining 22 microscopic fields of view (FOV) out of the near 120 of the human eye view.

So far more than 50,000 slides have been processed with a successful rate of more than 95.5%. The rejection rate (4.5%) is mainly represented by a heavy inflammatory component, irregular clotted mucoid spots, unsuccessful identification of specimens, vial labelling problems, mounting irregularities of the cover slip and misplaced slides within the imager grid. During the assisted imager phase, computer analyses selects 20 microscopic fields based on optical density of the nucleus, nuclear size and stain intensity, and 2 more fields based on cluster features. The main obstacles for cytotechnologists and cytopathologists are the Thin Prep Pap Stain, which is sometimes darker in metaplastic and glandular cells, and partial examination of the slide. Consequently, at the end of the first year of the project, 12 of 24 cytology staff are fully confident with the system, diagnosing negative cases relying only on 22 FOV scrutiny.

Helpful aspects in the changeover are a pertinent training program, regular and diligent application of the method, study and reliance on literature data and progressive self-confidence strengthened by positive feedback of results.

The philosophy of the new system relies on a positive relationship between man and computer carrying out a synergic action between two different intelligent forms, focusing on small isolated atypical cells or small groups of cells. Daily work is consequently made easier, allowing more rapid management of negative cases, quicker identification of abnormalities and increased efforts on difficult cases. Increased productivity is anticipated at the end of an appropriate training period as the consequence of reduced reading time.

In conclusion, if Pap test screening – right from the start – are looking for a needle in a haystack, we greatly appreciate Bruce Dziura’s statement that “the merge of mind and computer in the ThinPrep Imaging System has created a better Pap test”.

C1-C5 in breast cytology


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Breast fine needle aspiration cytology (FNAC) represents the diagnostic tool of choice to characterize mammographic lesions and clinically palpable breast nodules. The method is considered to bring advantages, in terms of low invasiveness and costs compared with other techniques like core biopsy or surgical biopsy, especially when it is performed under ultrasound guidance, which guarantees perfect targeting of lesions.

Most criticisms of FNAC are related to the different situations where the exam is performed and to the variety of breast lesions examined. The most reliable results with FNAC are obtained in those clinical groups where the pathologist, who is also a cytopathology expert, actively joins in aspiration
sessions and oversees collection of clinical data, and of radiological and ultrasound features of lesions. The pathologist can then evaluate the macroscopic characteristics of aspirated material (fluid, dense, whitish, jelly like, smelly, etc.) that will be spread on smears and evaluated immediately using a fast stain. The pathologist can evaluate the adequacy of the specimen and get an idea of the relevant clinical issues.

FNAC is influenced by a certain degree of subjectivity, that is more evident in the so-called “grey zone” cases (those not clearly benign or malignant) and when there some doubts about material adequacy. These situations may create some misunderstandings between clinicians and pathologists with possible over or undertreatment for patients. The need for clear communication is extremely important, but becomes a priority in breast lesions because of the multidisciplinary aspects of the field. For this reason, a breast cytology reporting system has been proposed since 1993 within a UK mammographic breast screening programme. The system’s aim is to communicate to clinicians in an extremely precise way the pathologist’s evaluation about cytological cases: Benign (C2); malignant (C5); Inadequate (C1); Atypia probably benign (C3); Suspicious of malignancy (C4). The five diagnostic categories proposed in the UK in 1993 were later adopted at a European level; using this system does not limit the pathologists’ ability to give a description of morphological aspects observed on smears, but it requires a conclusive short diagnostic assessment that all other breast screening unit members can undoubtedly understand to allow precise and exact indications regarding further exams or treatment to be given. Diagnostic categories have also revealed to be an extremely useful tool for all the auditing activities each pathology laboratory has to respect within an organized screening programme, providing periodic data that demonstrate respect of quality control. In fact, all the activity’s reports and correlations regarding the diagnostic efficacy of cytology are based on data obtained by diagnostic categories and by comparison of cytologic diagnosis with subsequent histology or clinical follow-up. Making use of the five diagnostic categories also permits easy exchange of information and comparison among labs in different regions. In this way, any abnormalities can be easily discussed and corrected.

We have introduced the five diagnostic category system to report breast cytology in our lab since 1995 in an experimental manner, but it has become an essential part of cytological reports since 2000. A mammographic breast screening programme, which has started in our region, Friuli Venezia Giulia, since 2006, has made this mandatory for all cytological reports of pathologists involved in the screening programme. This allows the Public Health Regional Agency (which controls and coordinates the programme) to monitor the diagnostic quality of cytology reports.

Each diagnostic category is strictly associated with a diagnostic pathway which needs justification for modification. C1, for example requires the exam to be repeated or histology which becomes mandatory for those cases with suspicious radiology; in case of dubious radiology, the patient can be also recalled early for instrumental follow-up. Since the pathologist is present during FNAC sessions, our inadequacy rate is very low; and besides the screening detected lesions, cytology has represented the first morphological exam for about 90% of women with breast abnormalities.

In Table I we have summarized all cases examined by FNAC in the first six months of 2006 and compared them with those examined in the first six months of 2008 (Tab. II); by comparison it can be observed that there is homogeneous distribution of cases and diagnoses which are representative of the performance of breast cytology in the Trieste area.

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<thead>
<tr>
<th>Tab. I. Data records 1/1/06-15/08/06.</th>
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<tr>
<td><strong>2008 Data</strong></td>
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<tr>
<td>Malignant histology</td>
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<td>Benign histology</td>
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<td>No histology</td>
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<th>Tab. II. Indicators.</th>
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<td><strong>No case</strong></td>
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<td>409 (2006 data)</td>
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<tr>
<td>419 (2008 data)</td>
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<td>Standard (percent value)</td>
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<th>Tab. III.</th>
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<tr>
<td>As = absolute sensitivity</td>
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<td>Cs = complete sensitivity</td>
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<tr>
<td>Sbx = specificity (biopsy cases only)</td>
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<tr>
<td>Spe = specificity (full)</td>
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<tr>
<td>C5 PPV = positive predictive value (C5 diagnosis)</td>
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<tr>
<td>C4 PPV = positive predictive value (C4 diagnosis)</td>
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<tr>
<td>C3 PPV = positive predictive value (C3 diagnosis)</td>
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<tr>
<td>Fis- = false negative rate</td>
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<tr>
<td>Fis+ = false positive rate</td>
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<tr>
<td>Ina = inadequacy rate</td>
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<tr>
<td>Inca = inadequacy rate from cancers</td>
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<td>Sus = suspicious rate</td>
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Quality indicators of breast cytology (sensitivity, specificity, predictive values; false positives and inadequates) have also been quantified for the two periods. All cytological diagnosis were compared with histology, and for those cases not requiring surgery, a comparison with clinical follow-up was made. From our experience, we believe only a cytological exam with well demonstrated and well documented quality, which satisfies criteria suggested by guidelines, can play a key role in clinical management (Tab. III). It will also allow a reduction in the number of diagnostic biopsies on benign lesions and of frozen sections to confirm malignant lesions.

References
*Guidelines for cytology procedures and reporting in breast cancer screening*. NHS BSP screening publication (Sheffield) 1993.
Errata

Reviewing morphometry in L.B.C.C.
for cervical cancer screening
S.A. Senatore
U.O.C. di Anatomia Patologica, P.O. Gallipoli

Corrige

Reviewing morphometry in L.B.C.C. for
cervical cancer screening
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(Italian Division of the International Academy of Pathology

XIX Annual Scientific Meeting of the Italian Group of Ultrastructural Pathology SIAPEC-IAP (GIPU)

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by selective degeneration and death of both upper and lower motor neurons, resulting in paralysis due to muscle weakness and atrophy. Generally skeletal myofibers are able to regenerate after injury, thanks to satellite cells (SCs), myogenic precursor cells located between the basal lamina and the sarcolemma of mature myofibers. In ALS patients the regenerative capacity of skeletal muscle is compromised. In order to study in more detail the molecular mechanism, the proliferative and differentiative ability of SCs, we isolated SCs from ALS aging human muscle biopsies and analyzed their morphology by TEM and ICC and their capacity to grow in vitro. Moreover RT-PCR were performed to evaluate the expression of MRFs. ALS SCs showed a high ability to proliferate, but their capacity to proceed through the myogenic program and actively form myotubes seems to be altered compared to the aging control samples. In addition we observed in vitro that differentiating ALS SCs display an altered morphology which could be linked to their impaired regenerative potential.

Role of the electron microscopy in the definition of so-called “vacuolar myopathies”

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Main histopathological changes in muscle biopsy include myofibrillar disorganization, with or without inclusion materials and vacuoles. These latter in muscle biopsy can be considered related to many diseases; as regards to morphological features, they may be pathognomonic morphologic hallmarks, suggestive changes, but also aspecific alterations. Moreover, vacuoles often appear morphologically unremarkable and thus they are not readily visible on light microscopy. Ultrastructural analysis allows us to identify two types of vacuolar changes: cytoplasmic areas without limiting membrane, such as vacuoles seen in lipid storage myopathy, non-lysosomal glycogen storage myopathy and diseases with “rimmed vacuoles”; membrane-surrounded vacuoles, are seen in inherited lysosomal disorders and myopathies with autophagic vacuoles. Electron microscopy, despite limitations related to the capacity of the cell to react in a stereotyped manner to different insults, can be considered a useful tool in identifying changes, pathogenic or not, which cannot be shown up by light microscopy. Moreover, electron microscopy gives insight on pathophysiological mechanisms and can guide molecular genetics analysis. In conclusion, electron microscopy is an interesting tool for study of myopathies, in particular used diagnostically, its main indications are both myofibrillar and vacuolar myopathies.

Ultrastructural study and therapeutic approach of a case of “Action myoclonus-renal failure syndrome (AMRF)”

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“Action myoclonus-renal failure syndrome (AMRF)” is a lethal inherited form of progressive action myoclonus associated with renal failure. Recently a mutation in SCARB2 gene has been identified as likely cause. SCARB2, having pleiotropic effect, encodes for LIMP-2 receptor which binds β-glucocerebrosidase in the endoplasmic reticulum. Neuropathologic examination of one AMRF patient revealed uncharacterized material accumulated in the brain. Moreover, recent studies in LIMP-2 knockout mice showed glucosylceramide stored in the liver and lungs while it was not detected in spleen, kidney or brain. By Electron Microscope we studied muscle and skin biopsies and cultured skin fibroblasts of a patient with AMRF and SCARB2 mutation manifesting renal symptoms. Muscle biopsy only showed predominance of type II fibers as histopathological alteration. At ultrastructural level: areas with prominent disarray, no storage inclusions except for vacuoles containing electron-dense lipofuscin-like material in the rare fibroblasts. Furthermore, lysosomal structures containing granular-electrodense, ceroidolipofuscin-like materials, Gaucher-like tubular structures, inclusions like cholesterol crystals were identified in skin biopsy fibroblasts. Finally, cultured skin fibroblasts showed many vacuolar structures containing myelin whorls and heterogenous storage materials. Our data, especially skin and cultured fibroblasts, suggested the different nature of inclusions so that we could associate AMRF to a miscellaneous storage disease.

Ultrastructural characteristics of early endothelial progenitor cells


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Recently, it has been demonstrated that vasculogenesis is not limited to embryogenesis, but also occurs in adults under both physiological and pathological contexts. The integrity of the vascular system is essential for supporting the entire body’s life. Chronic mechanical and physical stresses compromise many functional activities of the vascular wall; endothelial cells (ECs) are major targets of injuries and their dysfunction...
is linked to the onset of atherosclerosis. Vascular resident ECs can replace immediately neighboring damaged cells owing to normal physiology, but when more extensive injury occurs their contribute is limited and insufficient; in this case vascular progenitors from peripheral tissues are mobilized efficiently. Vascular injury, indeed, leads to blood release of cytokines and chemokines which first recall and activate endothelial progenitor cells (EPCs) and then support their homing from native to damaged sites. EPCs represent only a small subset of the whole blood mononuclear fraction where they can be mainly isolated through immuno-sorting procedures; it has been demonstrated that the EPC population includes cells which belong to different hierarchical stages ranging from the hemoangioblast to the fully differentiated EC; each category gives a different contribute to angiogenesis in vivo. Many studies divide EPCs in two groups, the early and late EPCs, according to the following criteria: time-dependent appearance of colonies in vitro, morphology, proliferation rate and survival features. The immunophenotype and in vivo function of these two EPC classes are also well studied; less is known about their ultrastructural features. Here we have investigated through transmission electron microscopy the subcellular morphological details of early EPCs isolated from healthy volunteers; successively, we have promoted their differentiation toward the endothelial lineage using the angiogenic factor VEGF and recorded any morphological change consonant with the phenotypic switch; the ultrastructural characteristics of fully differentiated endothelial cells, i.e., human umbilical vein endothelial cells (HUVEC) and CD34+ cells derived from the adipose tissue, were included for comparison. Endothelial commitment of early EPCs is associated with the development of complex surface membrane invaginations, cytoplasmic whorling, increased number of Golgi complex cisternae, endocytic vesicles and appearance of Weibel Palade bodies. The functional characteristics of EPCs make them good candidates for regenerative medicine and clinical practice although further studies are necessary; our results indicate that the ultrastructural analysis of EPCs can help investigators to fully exploit the identity of EPCs with overlapping immunophenotypes and molecular profiles; this is critical to select a subset of EPCs tailored to specific therapeutic requirements.

**Histological and ultrastructural methods for characterizing mesenchymal cell interaction with electrospun nanofibrous scaffolds**

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Tissue engineering is a rapidly growing multidisciplinary field of science which integrates the basic principles of biology and engineering with the aim to obtain biocompatible, biodegradable, biologically active scaffolds suited for tissue regeneration or replacement. In recent years the development of new methodologies has made possible the achievement of synthetic fibers of unusually small diameter; these nanofibers can be randomly layered to form 3-D architectures which simulate the extracellular environment of soft tissues; the resulting scaffolds have the potentiality to modulate and transduce the chemical and physical signals that are essential for promoting cell survival, migration, proliferation and differentiation. In this study, the interaction of mesenchymal cells (vascular wall mesenchymal stem cells [VW-MSC] and human umbilical vein endothelial cells [HUVEC]) with nanofibrous scaffolds made of acid poly-L-lactic (PLLA) has been investigated using complementary morphological techniques; the PLLA nanofibrous scaffolds were obtained through the electrosprinning technology; VW-MSCs were isolated from human femoral arteries whereas HUVEC derived from discharged umbilical chords; cells were seeded at appropriate densities on the substrate surface for at least 14 days under plain culture conditions; scanning electron microscopy was used to determine the degree of mesenchymal cell surface colonization and the modality of cell adhesion on the PLLA scaffold; immunofluorescence microscopy of DAPI stained whole mount samples was used to quantify cell adhesion on PLLA nanofibre surfaces; cell penetration in the scaffold thickness was initially evaluated using E&E stained cryosections at light microscopy; however, early attempts with this approach were poorly satisfactory due to methodological shortcomings such as difficulty in sample handling and poor image resolution; paraffin embedding of nanofibrous scaffolds was achieved thanks to some tricks, i.e., precise sample orientation and temperature control during paraffin infiltration; the histological method had the advantage to produce both “en face” and transversally cut sections that can be used to evaluate cell interaction, penetration and colonization with nanofibrous scaffolds effectively; this strategy makes also feasible the application of immunohistochemical techniques which are valuable tools to investigate the fine molecular mechanisms which are involved in the interaction of mesenchymal cells with polymeric nanofibres.

**Cell therapy for tissue repairment: application of pharmacologically active PLGA microspheres as carriers of mesenchimal (stem) stromal cells**


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Cell therapy and tissue engineering represent new promising strategies to repair diseased organs and tissues. However, their clinical application requires the overcoming of several ethical, biological, technical and procedural difficulties; in the matter of biological problems, for instance, it’s necessary to establish the survival period of the grafted cells, their commitment or differentiation grade and their integration mode in the host tissues. Growth factors may be employed to meet with these problems but their administration still remains a technological challenge, due to their short life and pleiotropic actions.
To ward off this limitation, a methodological site-specific delivery approach has been developed employing spherical particles, composed of PLGA [poly(D,L-lactic-co-glycolic acid)], as carriers. These Pharmacologically Active Microcarriers (PAM) of 1-90μm are biocompatible and biodegradable; being coated with adhesion molecules they may serve as support for cell culture and may be useful as cell carriers, presenting a continuous and controlled delivery of active proteins. In the present study we have characterized the microspheres in a three-dimensional manner, we have evaluated cells-PAM interactions and we have observed “in vitro” differentiation of the cells adhering to the PAM. To develop this tool, cultures of PAM and cells in suspension have been organized, using microspheres coated with fibronectin and mesenchymal stromal cells isolated from heart-beating multigorgan donor femoral artery (hMSCs). Therefore, ultrastructural evaluations by means of Transmission and Scanning Electron Microscopy (TEM and SEM) have been carried out. Evaluating by SEM a suspension of PAM, we have been able to establish that the microspheres are characterized by a porous surface and a variable size (1-3 μm). Four hours after seeding PAM and hMSCs in the culture plate, the cells were almost completely adherent to the PAM, as observed at the reverse light microscope and after 24 hours, hMSCs have formed spherical collections including the PAM. These large aggregates were shown by SEM evaluation too. After 3 days of culture, one of these aggregates was gently taken up from the plate and processed for the transmission electron microscopy. By TEM ultrastructural analysis demonstrated that hMSCs adhering to the PAM were viable with intact nuclei and prominent nucleoli; moreover, they appeared undifferentiated retaining their basic mesenchymal features, such as loosely dispersed intermediate filaments of vimentin-type and well developed rough endoplasmic cisternae. A few cells contained intracytoplasmic phagocytized PAM fragments, without showing signs of cell degeneration; this finding confirms that PAM are inert and non-toxic.

At the end of the observations which have been carried out, it’s obvious that hMSC from femoral artery tightly adhere to PLGA-based microspheres coated with adhesion molecules, giving origin to spherical multicellular aggregates; during the culture, hMSCs have retained their basic undifferentiated features and this finding constitutes a condition in favour of their “in vitro” differentiation. Nevertheless, the large multicellular aggregates formation represents the highest limitation for the study of system efficacy in the animal models.

**Methods.** In the present work we have tried to retrieve two different antigens on sections put on Formvar coated nickel grids. The tissues were fixed in a mixture of 2% paraformaldehyde and 2% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in Epon-Araldite resin. Two different heatig methods were employed: the microwave oven and the autoclave.

**Results.** Immunostaining for chromogranin A in gastric mucosa: We have seen an increase of the immunoreactivity employing a microwave oven at 600W for 15’ (5’X3), less marked employing citrate buffer pH 6, more marked employing EDTA buffer pH 10, the immunoreactivity was however much more enhanced employing an autoclave, at 126°C for 15’with EDTA buffer pH 10.

Immunostaining for the enzyme BACE2 in pancreas: In rat pancreas, employing the autoclave at 126°C for 15’ with EDTA buffer pH 10, we have seen a marked increase of the immunoreactivity and also a reduction of unspecific stainings.

**References**

Brorson S-H, Nguyen GH. Increased level of immunogold labelling of epoxy sections by rising the temperature significantly beyond 100°C in the antigen retrieval medium. Micron 2001;32:591-7.


**Thin glomerular basement membrane disease or Alport syndrome: intriguing cases**

P. Preda

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Thin glomerular basement membrane (GBM) disease is a heritable nephropathy clinically characterized by a persistent and asymptomatic haematuria without extra renal manifestations. The pathological hallmark is a diffuse thinning of the glomerular basement membranes at the ultrastructural level. Thin glomerular basement membrane disease and Alport syndrome can be considered genetic diseases involving the α3/ α4/ α5 of collagen IV network. Mutations of COL4A3-COL4A4-COL4A5 genes can lead to a total or partial loss of this network. The diagnosis of thin GBM disease is difficult, in part because of the wide range of GBM thickness in the normal population (sex, age, different method of tissue preparation and measurement). In addition, there are no standardized diagnostic criteria defining the degree of extent.
of GBM thinning. Moreover, the modifications of basement membrane in Alport disease sometimes mimic a thin GBM disease. The differential diagnosis between Alport syndrome and thin GBM disease because of its genetic, clinical, immunohistochemical and ultrastructural heterogeneity, still remains a diagnostic challenge: a correct diagnosis is important because of the very different prognoses of these two conditions. We reviewed 650 renal biopsies submitted to ultrastructural examination in our unit from January 2001 to December 2008. Thirty three biopsies showed GBM thinning of variable entity. Excluding biopsies from patients with IgA nephropathy (13) which is known to be frequently associated with thin GBM’s, 3 cases, respectively of membranous GN, SLE and minimal change nephropathies and 3 cases of Alport syndrome, 15 biopsies showed features of a thin glomerular basement membrane disease but generically diagnosed as hereditary nephropathies. No one of these cases were surely diagnosed only on the basis of both ultrastructural data and poor clinical data and family history, although electron microscopy is considered the more important tool for the diagnosis of these nephropathies. The diagnosis of thin GBM disease is not always easy and great care should be made to distinguish it from Alport syndrome especially in the early stages of the disease or in female patients where reduction of GBM thickness could be the only morphological data. The genetic analysis and the immunohistochemical staining of type IV collagen chains will be a valuable adjunct to the diagnostic workup of these patients. Patients with thin GBM should be submitted to an accurate follow-up in order to find clinical signs of disease progression such as worsening of renal function, a superimposed immunological nephropathy, an evolution in Alport syndrome and last but not least, the possibility of transmitting the autosomal recessive form of Alport syndrome.