New Trends in Pap Testing

Marluce Bibbo, M.D., Sc.D., F.I.A.C.

The conventional Pap test is highly effective for screening of cervical cancer and its precursors but has a false negative rate due to either sampling, preparation or screening. New technologies, such as liquid-based Paps, automated screening devices and high-risk (HR) HPV testing by molecular methods, have been introduced to increase the detection of cervical disease.

Two liquid-based technologies (LBTs) have been approved by the United States Food and Drug Administration: ThinPrep and SurePath. Other LBTs processed by manual methods, such as PapSpin, Cyto-Screen & DNA Citoliq, have been introduced in Europe and South America. The main advantages of LBTs are well-fixed preparations free of blood or inflammatory exudate, smaller area to screen, more sensitive than conventional cytology and residual material available for ancillary testing for HR HPV DNA, Chlamydia/Neisseria gonorrhoeae and biomarkers.

The potential clinical utility of HR HPV DNA testing includes the triage of women with ASC-US results, in the posttreatment of women with CIN 2–3 and as an adjunct to the Pap test in primary cancer screening in women 30 years of age or older.

These new technologies have been gradually introduced at Thomas Jefferson University Hospital and were well received by the clinical staff.

It has been shown in the literature that the use of new technologies detects more cervical disease, although degrees of variability exist concerning associated specificity.

At the conference, the 2001 Bethesda System for the reporting of cervical cytology, including criteria for the interpretation of cervical lesions in liquid-based and conventional preparations, will be presented. The impact of HR HPV testing in the management of patients with cytologic abnormalities will be discussed.

Professor of Pathology, Thomas Jefferson University, Philadelphia, Pennsylvania, U.S.A. (bibbo@cytology-iac.org).
George L. Wied and the Gospel of Cytopathology

Leopold G. Koss, M.D., F.I.A.C.

There is a striking similarity between two remarkable people who died within a few months of each other: Pope John Paul II and George Wied. Both were indefatigable world travelers spreading a gospel: the Pope of Catholicism to save souls and George of cytopathology to save lives.

This presentation will address the life and oeuvre of Dr. Wied as a leader of international cytopathology, educator, editor, scientist and colleague of many years. The story of TICAS, a pioneering concept of image analysis that led to many important developments in this area, will be summarized.

Chairman Emeritus, Department of Pathology, Montefiore Medical Center, Bronx, New York, U.S.A. (lkoss@montefiore.org).

Development of the IAC Cytotechnology Examination

Catherine Keebler, Sc.D.(hon), C.F.I.A.C.

Objective: To provide information to the audience on the history, development and purpose of the IAC Comprehensive Cytotechnology Examination.

Methods: Based on information obtained from archival documents written by Dr. Wied and from historic data related to the examination process, the audience will be introduced to methodologies used to develop and evaluate the microscopic portion of the examination.

Results: From the evaluation of one microscopic case, the difference in results between various cytotechnology groups will be demonstrated.

Conclusions: The importance of national and international standardized education programs that help produce well-educated cytotechnologist colleagues will be discussed.

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George L. Wied’s Impact on the Development of Gynecologic Cytology in Austria

Gerard Breitenecker, M.D., F.I.A.C.

In Austria cervical cancer mortality has been reduced by three quarters since the 1960s. During this time the number of smears increased from 250,000 to 1,750,000 annually. George L. Wied had substantial influence on this development and success. He arranged during three decades (1970–1998) of International Tutorials of Cytology in Vienna and made Vienna an international center of education in clinical cytology. With the help of sponsors it was possible for colleagues from Eastern European countries to come to Vienna and to attend the courses. Thus, Wied was a bridge builder, a pontifex in international cytology. George Wied’s outstanding talent and experience in organization of meetings and his readiness to help with his advanced computer system made it possible for us to arrange the European Congress of Cytology 1993 in Vienna and to publish the abstracts in Acta Cytologica.

Today an opportunistic screening system is in place in Austria and reaches approximately 60–70% of the target population. For further improvement in the mortality rate of cervical cancer in Austria the following efforts are being made:

Introduction of organized screening with recall system in 2005, obligatory reporting of the results of all laboratories to a Committee of Quality Assurance of the Austrian Society of Cytology (so far voluntary); reevaluation of preceding smears within the last five years in cases of invasive cervical cancer; guidelines for the management of women with suspicious and positive cytology in collaboration with the Austrian Society of Gynecology and Obstetrics. Most of these means of quality assurance were inaugurated by George Wied many years ago. He also predicted many developments in cytology years ahead.

Professor of Pathology, University of Vienna, Austria, and Past-President of the Austrian Society of Cytology (gerhard.breitenecker@univie.ac.at).

The European Federation of Cytology Societies: A Catalyst

O. A. Nasseem Husain, M.D., F.I.A.C.

Objective: To achieve equal opportunities between 5 Eastern and 15 Western European Societies of Cytology before the fall of the Berlin Wall (1990).

Methods: Alternation of meetings between East and West.

Results: Achievement of quality of practice.

Conclusions: Highly satisfactory result.
The early concept of the EFCS was the brain child of Dr. Paul Lopes Cardozo. In 1970 Dr. Jacques Jenny hosted a meeting to discuss this. Dr. George Wied suggested a European Branch of the IAC, which was rejected, and he then organized the Vienna Tutorials, so we got both. In Europe we felt the need for an equitable system that made for easier contact between the five Eastern versus 15 Western societies, as many of those in the East could not get visas or funding to come to the West. With the creation of the EFCS it was possible to give the Eastern societies preferential frequency. The way this was organized will be discussed, as will the development of cytology in Europe.

Consultant Cytopathologist (ret.) and Second Secretary of the European Federation of Cytology Societies, London, U.K. (xot33@dial.pipex.com).

The Role of Genetics in Cancer Development

Alain Verhest, M.B., Ph.D., F.I.A.C.

Cancer arises by accumulation of mutations, amplification or overexpression of critical genes normally involved in the process of proliferation. In the last 3 decades cytogenetic analysis of human tumors has revealed a nonrandom distribution of chromosome rearrangements resulting in genomic imbalances that cause cancer and determine the biologic behavior of the abnormal clones. Balanced, simple and disease-specific genetic anomalies are found in one third of leukemias and lymphomas and 20% of other mesodermal tumors, where they often appear as fingerprints for a specific tumor type. Because of very complex aneuploidies, epithelial cancers need more sensitive techniques than conventional banding karyotypes. Integration of viral DNA into host cell DNA is essential for cervical cancer development. It occurs in chromosomes at random and results in uncontrolled cellular proliferation, leading to aneuploidy. Therefore, DNA content analyzed by laser scanning cytometry offers some clues to the tumorigenic potential of low grade cervical lesions with abnormally high DNA content. The location of mutated or amplified sequences on chromosomes can be visualized using fluorescence in situ hybridization on interphase nuclei from smears or paraffin-embedded slides. Aneusomies and gene amplification, when specific, can be used as biomarkers. Comparative genomic hybridization (CGH) is an hybridization quantitative technique measuring the size of all chromosomal imbalances within the tumor genome. In array-CGH, metaphase chromosomes have been replaced by spots of cloned DNA. This technique increases the resolution from a cytogenetic level to a molecu-
lar level, defining, by the application of gene expression profiling, a specific molecular fingerprint of chromosomal imbalances in tumors. Beyond HPV integration, the progression from precursor squamous intraepithelial lesions to cervical carcinoma requires additional genetic and epigenetic alterations that have not been fully characterized. However, our understanding of the pathogenetic alterations of malignant diseases already offers powerful diagnostic markers, prognostic indicators and promising tools for validating the concept of molecular targeted therapies.

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E-Learning in Cytology: Web-Based Training on Glandular Cells in Gynecologic Cytology with Collection of CME Credits

Ulrich Schenck, M.D., F.I.A.C.

Objective: The aim of this study was to implement web based training (WBT) to offer a course on “Glandular Cells in Gynecologic Cytology,” including online continuing medical education (CME) credit points according to the regulations of the Bavarian Medical Association.

Methods: Based on the user administration of a website with a multilingual image gallery (free registration) (http://www.zytologie.de), a concept was developed to offer online cytologic training courses. The WBT platform was programmed externally in php script language in a linux software environment and is running on remote web servers. It has been implemented in a way that user administration of image gallery and WBT is closely linked. User, trainer and administrative access is via web browsers and optimized for use with Microsoft Internet Explorer 5.5+

Results: (http://zytotraining.schenck.de) Users and trainers have access to the WBT by the same user name and password as for the image gallery. Administration takes care of user administration and regulates trainers’ rights. Access is highly individualized. The WBT main user surface is available in English and German. Functions of the home page vary with the selection of the courses. IA symbol-oriented navigation surface allows training in different languages according to the availability of teachers. A WBT on “Glandular Cells in Gynecological Cytology” has been implemented including more than 100 documents, over 200 images and several examination blocks consisting of a minimum of 10 questions of various types. Continuing education credit points are given according to the regulations of the Bavarian Medical Association based on participation according to different learning objectives, time and activity measurement, and evaluation in online tests. The course is in German for registrants of the Munich Postgraduate Courses of Cytology. All registered users of the image gallery have free but limited access to the training platform.

Conclusions: A WBT with CME credit in cytology, including monitoring participation and success to calculate credit points, has been developed and implemented. It is planned to improve multilingual functions and make courses also available in other languages.

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Glandular Cytopathology of the Female Genital Tract

Matías Jiménez-Ayala, M.D., F.I.A.C., and Beatriz Jiménez-Ayala, M.D., M.I.A.C.

The glandular lesions of the female genital tract are discussed following the 2001 Bethesda system for the various glandular epithelial cell abnormalities.

A. Benign glandular lesions.

Endocervical polyps.

Endometriosis: vulvar endometriosis, vaginal endometriosis, cervical endometriosis, fallopian tube endometriosis and adenomyosis.

Microglandular hyperplasia.

Vaginal adenosis.

Vulvar papillary hidradenoma.

B. Atypical glandular cells (AGC).

The features of glandular cells suggest neoplasia, but not all the criteria of adenocarcinoma in situ (AIS) or invasive adenocarcinoma are present.

C. Malignant glandular lesions.

Cervix:

In situ and invasive endocervical adenocarcinoma.

Infrequent tumors: glassy-cell carcinoma, adenosquamous carcinoma, carcinoïd and villoglandular adenocarcinoma.

Endometrial adenocarcinoma.

Extraterine adenocarcinoma.

Ovarian, fallopian tube and clear-cell adenocarcinoma.

Bartholin’s gland adenocarcinoma.

Metastatic adenocarcinoma.

President, International Academy of Cytology, Madrid, Spain (jimenezayala@cytology-iac.org).
Cytologic Diagnosis of Tumors of the Uterine Corpus


Objective: To evaluate the cytologic features of endometrial carcinoma, sarcoma and carcinosarcoma and their differential diagnoses.

Methods: Aspirates from the uterine cavity and touch imprints of resected primary and recurrent tumors and their metastases were studied. As techniques we used routine Leishman method, CellPrint, Cytospin-3 (Shandon, UK) and DNA flow cytometry (Epics-XL, Beckman-Coulter; MultiCycle software, Phoenix Flow Systems, USA). All cases were histologically confirmed. The material consisted of 159 endometrioid adenocarcinomas, 5 serous carcinomas, 6 clear cell carcinomas, 5 mixed adenocarcinomas, 1 mucinous carcinoma, 3 squamous cell carcinomas, 2 low grade stromal sarcoma (ESS), 4 high grade stromal sarcomas, 9 leiomyosarcomas, 1 rhabdomyosarcoma, 20 carcinosarcomas and 1 lymphoma (large, B cell type).

Results: The greatest difficulties arose in the diagnoses of well-differentiated endometrioid adenocarcinoma, low grade ESS and low grade leiomyosarcoma because of lack of cellular atypia. In imprints of smooth muscle tumors there were difficulties with the significance of atypical cells occurring in bizarre leiomyomas and their differentiation from leiomyosarcoma. In carcinosarcoma there were occasional problems with correctly identifying the mesenchymal component of the tumor. In the homologous variant the differential diagnoses includes high grade ESS and leiomyosarcoma and in the heterologous variant, rhabdomyosarcoma. It should be remembered that carcinosarcoma may give rise to metastases of both the epithelial and the mesenchymal components; only one component may then be present in the cytologic smear.

Conclusions: In routine imprints, cell prints and cytospin smears, endometrial carcinoma, sarcoma and carcinosarcoma can be diagnosed in most cases cytologically. Flow cytometry allows characterization of tumor cell populations and may give some prognostic information.

New Molecular Markers as Predictors of Viral Events and Disease Outcome in HPV-Associated Cervical Carcinogenesis: Experience from the HPV-PathogenISS Study

Kari Syrjänen, M.D., Ph.D., F.I.A.C.

Objective: Oncogenic HPVs are capable of contributing to the development of malignant phenotypes by several different mechanisms, most of which seem to be closely interrelated. Because of the fact that these molecular interactions are mediated by proteins, the logical strategy to dissect the complex molecular pathways is to study the functions of these proteins, utilizing immunohistochemistry (IHC).

Methods: In the ongoing HPV-PathogenISS project (Italy), we will target the key molecular pathways in cervical carcinogenesis using IHC-based strategies. A series of 302 archival samples, including 150 squamous cell carcinomas (SCCs) with complete follow-up data, and 152 CIN lesions (followed-up by serial PCR after treatment), were subjected to IHC staining with 13 different antibodies: p16INK4a, E-cadherin, MMP-2, TIMP-2, VEGF-C, nm-23 H1, laminin receptor, PCNA, NF-kappab, Topo-2a, telomerase, Survivin and ERK-1. Our aim is to assess whether any of these biomarkers might be useful in predicting several of the intermediate end point markers of cervical cancer: a) the grade of CIN, b) HR HPV type, c) clearance of the virus after eradication of CIN, or d) prognosis of cervical cancer.

Results: At this writing, results are available on 4/13 of...
these biomarkers (p16\(^{INK4a}\), ERK-1, survivin and VEGF-C) (to be discussed). Highly divergent molecular mechanisms neatly explain the different associations of HR-HPV and CIN discovered in these studies.

**Conclusions:** Following the completion of all 13 markers analyzed individually, the final step will be to analyze all 13 markers by multivariate modelling. Apart from getting new insights in the molecular pathogenesis of HPV-associated cervical carcinogenesis, we anticipate to disclose 1) individual markers, 2) a set of markers, or 3) an expression profile of any such marker sets that would be of clinical value as predictors of disease outcome in cervical carcinogenesis.

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### E6/E7 RNA Transcripts as a Predictor of Cervical Neoplasia

**Björn Hagmar,** M.D., Ph.D., M.I.A.C.

Detection of E6 and E7 mRNA transcripts has been shown to be of higher prognostic value for the evaluation of the precursor lesions of cervical carcinoma than the detection of HPV DNA in a number of pilot studies.\(^1\) In particular, in low grade lesions, HPV DNA testing has poor discriminating power as to the progression of CIN, thus leading to considerable overtreatment, with ensuing costs to the health care system. Available testing systems, molecular mechanisms and clinical studies will be presented and critically evaluated.


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### HPV E7 Oncoproteins as Potential Molecular Markers for Cervical Cancer Screening

**Barbara Fitzky,** Ph.D.

Epidemiologic studies have shown a clear correlation between high-risk HPV infections and the development of cervical and anogenital cancers. Persistence of the HPV infection by integration of the viral DNA into the host cell genome followed by a loss of transcriptional repression of the viral early genes E6 and E7 is known to be the causative agent for HPV-induced tumor development. We investigate whether the detection of high-risk HPV E7 oncoprotein might be a useful molecular tumor progression marker for the identification of HPV-induced high grade squamous intraepithelial lesions (HSIL) and early invasive cervical cancer since no E7 protein should be detectable in patients with transient HPV infections. Added to cytologic and HPV test-
The molecular differentiation of carcinoma of the ovary and peritoneal mesothelioma

Carlos Bedrossian, M.D., Ph.D. (hon), F.I.A.C., Ben Davidson, M.D., Ph.D., and Claire Michael, M.D.

Despite certain shared histogenetic characteristics, the cytopathologic distinction between ovarian carcinoma and peritoneal malignant mesothelioma (MM) may be at times very difficult. Ancillary methods that aid in this differential diagnosis are well established. However, little is known about the biologic characteristics that differentiate these two cancer types. We performed a comparative analysis of cancer-associated molecule expression in a cohort representing a total of 301 ovarian carcinomas (only peritoneal lesions) and 32 peritoneal MM. The molecules analyzed included nervous growth factor receptors (p75, p-TrkA) angiogenic factors (VEGF, IL-8, bFGF, heparanase), laminin receptors (67-kd receptor and the alpha6 integrin subunit), proteases (MMP-2, kallikrein 4), immune response mediators (HLA-G) and signaling molecules (the MAPK members ERK, JNK and p38). Various combinations of immunohistochemistry, western blotting and RT-PCR were applied to effusion and/or resected specimens and results statistically analyzed. MM specimens showed significantly higher expression of p75 (p < 0.001), p-TrkA (p < 0.001) and bFGF (p < 0.001) and significantly lower expression of the 67-kd receptor (p < 0.001), alpha6 integrin subunit (p = 0.023), VEGF (p < 0.001), IL-8 (p < 0.001), kallikrein 4 (p = 0.003) and HLA-G (p = 0.038) as compared to ovarian carcinomas. MM specimens showed higher activation ratio (phosphorylated/total enzyme ratio) of all three MAPK members (ERK: p < 0.001; JNK: p < 0.001; p38: p = 0.007) as compared to ovarian carcinomas. These data document significant differences in the expression of cancer- and metastasis-associated molecules in MM as compared to ovarian carcinoma; 2) suggest that different biologic pathways are involved in tumorigenesis and disease progression in these two tumors; and 3) offer some hope that detecting cancer-related molecules in cytologic samples may one day become a noninvasive means of selecting molecular targets of novel therapies for peritoneal MM and ovarian carcinoma.

Robert Leif, Ph.D., P.M.I.A.C.

Objective: Cytology automation will be enhanced by the creation of a common data format, such as the Laboratory Digital Imaging Project (LDIP) Data Exchange Specification (http://www.ldip.org/), which is being developed by the Association for Pathology Informatics. An international, vendor-supported, nonproprietary specification will allow pathologists and researchers to develop and use image capture/analysis software without worrying about incompatibilities between proprietary vendor formats.

Executive Officer, Amynon Biotech Company, Innsbruck, Austria (barbara.fitzy@amynon.com).
Methods: A committee composed of image vendors, academics and service-oriented pathologists is developing the specification in an open manner, which permits peer review. This committee is actively seeking other groups to join in this effort. The specification will consist of a collection of XML schemas that define common data types, elements and attributes, which, wherever possible, will be based on existing standards.

Results: The feasibility of creating the LDIP schemas was demonstrated by the previous creation of Cytometry ML, which includes schemas that describe instruments, staining and data. Many of the data types are based on Digital Imaging and Communications in Medicine (DICOM). Binary files for images and list-mode data have been created and read. Multicolor images with white backgrounds have been created from multiple monochrome fluorescence images. The interpretation by pathologists and other medical personnel of these multicolor fluorescence images should be facilitated because of their similarity to conventional absorbance images.

Conclusions: A common data specification for digital microscopy and flow cytometry can be created in a manner consistent with its use for medical devices and interoperability with both hospital information and picture archiving systems. This specification will allow pathology images and accompanying annotations to be exchanged and facilitate the creation of software by third parties.


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Automation of Pap Smear Screening: One Approach

Paul N. Holt

This presentation will provide an overview of the FocalPoint™ Slide Profiler technology and show FDA approval data as well as published results from a variety of sources.

Automated analysis of gynecologic specimens has been an area of high focus over the last 15 years. Today two products have obtained FDA approval. In this presentation the FocalPoint™ Slide Processor (TriPath Imaging, Inc., Burlington, N.C., USA) will be explained from the perspective of workflow and internal design characteristics. The FocalPoint™ has been in commercial use since 1995, when it was first introduced as a quality control rescreening device for conventionally prepared slides. In 1998 the system was approved by the U.S. FDA for primary screening of conventionally prepared slides with subsequent approval in 2001 for the processing of SurePath® liquid-based slides. The FocalPoint™ product integrates many state of the art subsystems to achieve its results. Multiple field of view computers provide high throughput and an essential built in back-up system to enhance reliability. A high precision motion system includes eleven motors capable of micron level repeatability and positioning. Two separately trained decision algorithms provide the intelligence in the FocalPoint™ slide analysis process. The combination of these components results in an automated, highly reliable system that has been shown to significantly improve a laboratory’s performance and efficiency when implemented in routine use. Today there are over 170 systems in place around the world, including Europe, Asia and North America.


Imaging of the Pap Test: Built on the Shoulders of a Giant

James Linder, M.D., M.I.A.C.

“If I have seen further, it is because I have stood on the shoulder of giants.” This comment was penned by Sir Isaac Newton in a letter to fellow English scientist Robert Hooke. Sir Isaac’s observation, written on February 5, 1675, could be written by thousands of pathologists and cytoengineers to acknowledge the vision they gained from Dr. George Wied. Nowhere is this more true than for researchers who spent their career developing computer-based imaging systems for the analysis of the Pap test. In research spanning more than 40 years, Dr. Wied and his colleagues developed the fundamental scientific basis for modern imaging technologies. This presentation briefly highlights Dr. Wied’s contributions related to spectrophotometric analysis of DNA and ploidy measurement as a basis for modern imaging methods exemplified by the ThinPrep Imaging System.

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Breaking Through the Open Door: Papanicolaou Smear in the 21st Century

Leopold G. Koss, M.D., F.I.A.C.

Programs based on universal access to the Papanicolaou
smear reduced the rate of cervix cancer by over 80%. Over the years, many papers pointed out the failures of the procedure. These voices were ignored until an article by Walt Bogdanich in the Wall Street Journal in November 1987 pointed out that women were dying of cervix cancer through laboratory errors. The cascade of events following this article led to numerous changes in the practice of gynecologic cytology. Innovations by collection of cells in liquid media and machine processing were introduced. Testing for HPV has become another step in cervix cancer detection. These procedures vastly increased the cost of screening, and so far there is no evidence that they had a major impact on the cervix cancer rate.

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International Tutorials of Cytology

The International Tutorials of Cytology were inaugurated in 1970 by Dr. George L. Wied and his team at the University of Chicago and quickly became legendary. The setup followed the already-established tutorials held in Chicago and Los Angeles since 1965. The program was structured as a weeklong, intensive course of clinical cytology of all body sites presented by an outstanding international faculty. There was a mix of lectures, workshops and panel sessions covering all topics and sites of clinical cytology, including fine needle aspiration, in a comprehensive fashion. The schedule was rigorous, often extending to 10 p.m. Attendees were pathologists, clinicians and cytotechnologists from numerous European and other countries. In all, over 4,000 practitioners have taken part in this exemplary educational event.

As shown in the following list, the majority of the tutorials, 13 of 22, were held in Vienna, at the Intercontinental Hotel. Other locations were Tokyo, São Paulo, Sidney and Prague.

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