SS-31  CERVICAL CANCER CONTROL IN CHILE: THE NATIONAL CYTOLOGY LABORATORY NETWORK

R. Prado

OBJECTIVE: Evaluate performance of the national cytoology laboratory network as related to requirements of the Cervical Cancer Control Program in Chile.

METHODS: Laboratory organization was evaluated and results during the period 1993–2005 were analyzed.

RESULTS: Screening was performed at 22 laboratories nationwide, with a total capacity of 800,000 Pap smears annually. A national reference laboratory provides for external quality assurance and the maintenance of a uniform laboratory-based information system. A total of 7,726,211 Pap smears were reported during 2005, with a positive rate of 2.1%; atypical squamous cells of undetermined significance (ASCUS)/atypical glandular cells of undetermined significance (AGUS) 1.3%; satisfactory but limited by absence of transformation zone components 13.4%; unsatisfactory 3.9%. A total of 10,364 women had biopsies because of atypical and positive cytology or clinical suspicion of cervical neoplasia. Cytologic-histologic correlation was 67% for low-grade squamous intraepithelial lesion (LSIL), 73% for high-grade squamous intraepithelial lesion (HSIL), and 78% for combined HSIL and invasive cancer. Sensitivity and specificity (ASCUS+) were 90% and 60%, respectively. Coverage of the population at risk increased from 26% in 1990 to 66% in 2005. Mortality for cervix cancer decreased in the same period from 12 in 100,000 to 7 in 100,000.

CONCLUSION: The capacity of the cytotherapy laboratory network is sufficient to cover 80% of the female population at risk, with a Pap test every 3 years. There is space to improve efficiency of conventional cytotherapy by selecting the most adequate devices for cell collection and providing continuing education to professional and technical personnel involved in sample collection and screening. No consideration has been given as yet to alternative screening methods such as liquid-based cytotherapy, the cost of which would increase 3–4 times the overall cost of the screening program.

SS-32  IMPORTANT ISSUES IN THE ORGANIZATION AND EVALUATION OF CYTOLOGY CONTROL PROGRAMS

L. Fernández

Cervical cancer is preventable, but it is still the most common cancer among women in Central American and many South American countries. Incidence and mortality trends have declined in many European countries, the United States, Canada and others. Many of these changes are due to changes in hygiene habits and sexual and reproductive patterns, but the effect of organized screening programs is not negligible. In Latin America, prevention efforts have focused mainly on screening women with cytology (Pap smears) through family planning services and treatment for neoplastic lesions, but organized programs are not in practice frequently. Thus most of these programs have resulted in an inefficient use of resources with no impact on incidence and mortality from the disease. Several countries, namely Brazil, Chile, Costa Rica, Cuba, Ecuador, El Salvador and Peru, are working to improve the effectiveness of cervical cancer screening. Intermediate indicators to evaluate the program process are very useful, in order to correct deviations and to explain the lack of success: screening facilities; the designation of special laboratories to provide cytotherapy services; the mechanisms for processing of cytologic smears and the return of results in an adequate time interval; the definition of referral mechanisms for patients; the creation of treatment centers for early or late stage lesions; the creation of an information system that allows evaluation process are some of requirements for these programs. The quality of the cytotherapy is a main determinant of a successful cervical cancer screening program. Laboratories should following international standards, and a laboratory quality control system should be in practice. The planning of management, supervision and systematic evaluation of the program is an important contributor. The collaboration with the cancer registry and mortality system is essential to have main indicators of results and impact of the program.

PLAT FORM PRESENTATIONS

HUMAN PAPILLOMAVIRUS (HPV)

A-01  A PEER COMPARISON PROGRAM FOR THE QUALITY ASSURANCE OF HPV DNA DETECTION USING THE DIGENE HYBRID CAPTURE II/SUREPATH METHOD SHOWS EXCELLENT ANALYTIC INTERLABORATORY CORRELATION

D. Kuebler, A. Illingworth, A. Bhane and D. Wilbur

OBJECTIVE: Interlaboratory peer comparison programs are a CLIA-mandated and integral quality assurance activity in the clinical laboratory. No commercial program is currently available specifically designed for cytotherapy laboratories performing HPV DNA (and no other types of viral) testing. We report the results of a self-developed program between 2 cytotherapy laboratories.

MATERIALS AND METHODS: Between 4 and 11 SurePath (TriPath) liquid-based cervical cytotherapy samples are selected at each of the 2 participating laboratories each quarter and exchanged without accompanying patient information. Samples are selected to test both positive and negative HPV DNA results in roughly equivalent numbers. Samples are run with the Hybrid Capture II (Digene) method using each laboratory’s standard procedure. The results are compared with the originating laboratory’s result. Statistics on correlation are compared on an ongoing basis as a method to assess each laboratory’s analytic performance.

RESULTS: Over a 3-year period, 12 exchanges took place, constituting 113 total specimens. Overall, there were 9 exchanges (76 specimens) showing 100% correlation and 3 exchanges (37 specimens) showing a total of 4 discordant results. Overall, this represents a 97% (109 of 113) correlation of results between laboratories. All 4 discordant cases were reported as negative by the original laboratory and positive by the exchange laboratory (2 in each direction).

CONCLUSION: The interlaboratory peer comparison result of 97% concordance shows excellent analytic agreement between the HPV DNA detection procedures of each laboratory. All discordant cases were “negative to positive” and were distributed equally by laboratory of origin.

From Massachusetts General Hospital, Boston, Massachusetts; and Dahl-Chase Diagnostic Services, Bangor, Maine, U.S.A.
A-02 THE FINAL RESULTS OF THE ARTISTIC TRIAL: A RANDOMISED TRIAL OF HPV TESTING AND PRIMARY CERVICAL SCREENING


OBJECTIVE: To evaluate the effectiveness of human papillomavirus (HPV) testing in primary cervical screening.

STUDY DESIGN: A prospective trial in which women undergoing primary cervical screening with liquid-based cytology were randomized 3:1 either to have the HPV result revealed and acted upon or to have the HPV concealed from the women and the health care providers. Women were then rescreened at 36 months with cytology and HPV testing. The study took place at Greater Manchester and involved attendees at routine National Health Service Cervical Screening Program screenings. Participants were 24,510 women aged 20–64. HPV testing was performed in primary cervical screening using the Hybrid Capture 2 (Digene) test. The primary outcome is the difference in cervical intraepithelial neoplasia (CIN) 3 incidence at 3 years between the 2 arms. Other outcomes were assessment of HPV as a standalone primary test with cytology as triage, outcomes in cytologically negative HPV-positive women and cost effectiveness of HPV testing.

RESULTS: The first round of screening took place between July 2001 and September 2003 and a second round began in July 2004 and will close at the end of October 2006 with around 15,000 having been rescreened in the second round.

CONCLUSION: A final analysis will be completed in the first quarter of 2007 and the final results of the clinical trial will be presented.

From University of Manchester, Manchester; London School of Hygiene and Tropical Medicine, London; Central Manchester and Manchester Childrens University Hospital National Health Service Trust, Manchester; and Brunel University, London, U.K.

A-03 DETECTION OF HUMAN PAPILLOMAVIRUS (HPV) HIGH-RISK DNA TYPE L1 CAPSID PROTEINS IN LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS AND HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS: HPV DNA–POSITIVE IMMUNOCYTOCHEMISTRY STAINED SPECIMENS HAS A PROGNOSTIC RELEVANCE

W. Steinberg, S. Tiews, C. Hanrath and R. Hilfrich

OBJECTIVE: Human papillomavirus (HPV) infection is associated with the development of precursor lesions and invasive cervical cancer (CC). Most of the infections are transient, and the multidimensional way of the cancer genesis is not completed. Cancer screening demonstrated effectiveness in Germany. Nevertheless, there exists an uncertainty about which morphologic suspect cases (low-grade squamous intraepithelial lesion [LSIL]/high-grade squamous intraepithelial lesion [HSIL]) of women who are infected with high-risk HPV types will demonstrate a progression or regression. Therefore we analyzed the prevalence of HPV DNA–L1 capsid proteins in LSIL and HSIL HPV DNA–positive smears by using the immunocytochemical HPV-L1 staining method (Cytoactiv). For detecting HPV DNA, the Hybrid Capture II Test (HC2T) was used. The L1 capsid protein is deemed to be a major target of cellular immune response in cervical intraepithelial neoplasia (CIN).

METHODS: The prospective study analyzed 151 routinely stained specimens (LSIL/HSIL) that were positive for high-risk HPV DNA with HPV DNA–L1 specific monoclonal antibody. Follow-up smears were scheduled in intervals of 3–6 months. Conization was performed if clinically indicated.

RESULTS: Thirty samples had to be removed from the study (lost to follow-up, pregnancy, staining error). Thirty-five were L1 positive. Histology approved 2 progressions (2 HSIL), whereas 20 cases of the L1-positive group demonstrated a regression. Thirteen cases showed stable disease. The remaining 86 specimens were L1 negative. Histology confirmed 31 progressive cases (31 HSIL), whereas in 28 cases a progression could be detected. However, 44 of them need reevaluation. Twenty-seven cases showed stable disease.

CONCLUSION: Using Cytoactiv, an HPV L1-specific monoclonal antibody, in LSIL and HSIL, specimens seem to be a suitable diagnostic tool to predict the prognosis of CC. In contrast to 31 L1 negative, only 2 L1 positive HPV DNA–positive LSIL/HSIL showed a progression. This strengthens the idea that L1-positive specimens are more likely to regress than L1-negative ones.

From the Laboratory of Cytopathology, Kloster Paradiese; and Cytoimmun, Pirmasens, Germany

A-04 MULTIPLE HUMAN PAPILLOMAVIRUS GENOTYPES IN CERVICAL SAMPLES OF DUTCH WOMEN PARTICIPATING IN THE NATIONAL SCREENING PROGRAM

M. Boon, L. Boon and H. Korporaal

OBJECTIVE: Multiple human papillomavirus (HPV) type infections have gained increased attention mainly because of the development of prophylactic vaccinations. Few aspects of the occurrence of infection with multiple HPV types have been described in Europe. The goal of the study was to document the prevalence of cervical coinfection with multiple HPV types in Dutch women participating in the national screening program and to assess if particular HPV genotypes are more or less likely to be involved in multiple infections.

MATERIALS AND METHODS: Asymptomatic women were invited for a screening test in the National Screening Program. Cases in which koilocytes were detected (in the ThinPrep slide) were tested for 26 HPV genotypes. The classification was in the majority of the cases atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesions (LSIL). The cervical samples chosen were suspended in BoonFix, and the cell sample left over in the vial (after the ThinPrep slide was prepared) was used for polymerase chain reaction (PCR). The short fragment PCR (SPF) hybridization line probe assay (LiPA) was used.

RESULTS: The most frequently encountered high-risk HPV was HPV 16 directly followed by HPV 51. Of the 938 positive tested women, 281 harbored multiple HPV types, some as many as 6 different genotypes. Twenty percent of the HPV-positive cases had more than 4 different HPV subtypes in the sample. We observed more frequently occurring coexistence of other viruses and HPV 16 or HPV 51 than single HPV 16 or HPV 51 in the group of 3,303 women studied.

CONCLUSION: Dutch women harbor more frequently multiple papillomavirus genotypes than might be expected. The implications of this finding for prophylactic vaccination in the Netherlands has yet to be validated in a larger series.
A-05 THE EFFECTS OF THE FIVE YEARLY DUTCH SCREENING PROGRAM: HUMAN PAPILLOMAVIRUS AND (PRE)NEOPLASIA

M. de Bosschere, M. Boon, L. Boon and L. Kok

OBJECTIVE: Two screenings of the Dutch 5-yearly cervical screening program are completed, and thus screening with this relatively large interval can be evaluated. In this program, women are invited for a screening test without costs. Screening outside this program is discouraged, resulting in a high participation and < 10% interval smears. The goal of the study was to document the changes in prevalence of active human papillomavirus (HPV) infection (koilocytosis) and squamous intraepithelial lesion (SIL) in 6 birth cohorts.

METHODS: The database for this study consisted of over 400,000 cytologic diagnoses of over 200,000 women. Cohort 1 was born in the years 1965–1969 and cohort 6 in the war years 1940–1944. The scores assessed in the second screening were corrected for each of the 6 birth cohorts. The corrections were based on the mutation in women 3 years older as found in the first screening, and varied from 0 to 62%, the highest correction being for koilocytosis in cohort 5.

RESULTS: For koilocytosis, the increase in prevalence by 3-yearly screening increased for the cohorts 1–4, with a doubling of the scores for the youngest cohort. For preneoplastic low-grade SIL (LSIL), the prevalence decreased in all cohorts. For (pre)neoplastic high-grade SIL (HSIL), the patterns were fundamentally different, with no changes in the scores for all 6 cohorts.

CONCLUSION: Screening with a 5-year interval did not result in any change of the scores for HSIL, indicating that a balance is reached between progression and regression. In contrast, the significant increase of the scores for koilocytosis in the younger cohorts might indicate an increased rate of active HPV infection in the past 5 years.

A-06 AN EPSIN-DEFICIENT CELL LINE: A POWERFUL TOOL TO INVESTIGATE CLATHRIN-MEDIATED HPV INTERNALIZATION

C. Horvath, G. Boulet, D. Vanden Broeck, S. Sahebali and J. Bogers

OBJECTIVE: The human papillomavirus (HPV) is acknowledged as the major etiologic factor for malignancies of the anogenital tissue. To induce its pathogenic effect, HPV must be actively internalized by the cells of the anogenital epithelium. The manner of HPV internalization has already been extensively studied, and different endocytic pathways have been proposed. It is unclear whether functional endocytosis of HPV occurs via lipid rafts or caveolae or via clathrin-mediated endocytosis. The recently developed technology of RNA interference (RNAi) was exploited to suppress the expression of epsin, an essential component of clathrin-mediated uptake. The aim of this study is the development and characterization of an epsin-deficient cell line that can be used as a powerful tool to investigate the internalization mechanisms of different pathogens, more specifically, HPV.

MATERIALS AND METHODS: Transformation of the Caco-2 and HeLa cell lines with a vector expressing siRNA, targeting a well-conserved region of the epsin-encoding mRNA, resulted in a cell line with a depleted epsin expression as confirmed by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and Western blot. Cell morphology and transfection efficiency were microscopically evaluated. In order to control the effect of reduced epsin expression, a transferrin internalization assay was performed that showed reduced uptake in epsin-depleted cells.

RESULTS: No differences in cell morphology was observed between control and transfected Caco-2 cells. However, microscopic evaluation of siRNA-treated HeLa cells showed morphologic changes, which are clearly distinguishable from liposome-induced cytosolic side effects.

CONCLUSION: HPV infection plays an important role in the development of anogenital malignancies. In order to prevent HPV infections it is essential that the viral uptake mechanism is clearly identified. Since HPV internalization is an important step during the infection process, it is the ideal point of interfering with the viral lifecycle progression. The epsin-deficient cell line can be used as a general and powerful tool to investigate clathrin-mediated pathogen-uptake by human cells.

A-07 THE PRESENCE OF HRHPV AND OPTIMAL VISUALIZATION OF P16INK4A OVEREXPRESSION IN CERVICAL SAMPLES

E. Ouwerkerk-Noordam, F. Ghoubari, H. Korporeal and M. Boon

OBJECTIVE: The p16INK4a tumor suppressor protein, related to human papillomavirus (HPV) infection, has been reported to play a critical role in cell cycle regulation in cervical cancer; however, the p16INK4a immunostaining evaluation on cytologic slides is far from simple. The aim of this study was to develop an optimal p16INK4a staining on paraffin sections prepared from cytoblocks and compare the p16INK4a overexpression with the presence of hrHPV.

MATERIALS AND METHODS: All screening samples are received in the laboratory in a vial containing BoonFix, using the T3000 to prepare ThinPrep slides. We selected 119 cases on which HPV detection using the LiPA PCR method was performed on the same sample. From the material left in the vial a Shandon Cytoblock was made, with the method developed in our laboratory using microwave technology (Milestone). Sections were cut, and the p16INK4a method was performed using the CINtec p16INK4a Histology Kit on the paraffin sections.

RESULTS: Of the 119 samples, 64 had normal cytology, 6 were diagnosed as atypical squamous cells of undetermined significance (ASCUS), 22 as low-grade squamous intraepithelial lesions (LSILs) and 27 as high-grade squamous intraepithelial lesions (HSILs). The p16INK4a staining was excellent, allowing precise evaluation of the staining patterns. Of the 119 samples, 79 were hrHPV+ and 56 were p16INK4a+.
A-08 THE USE OF P16INK4A CYTOLOGY FOR DETECTING HIGH GRADE INTRAEPITHELIAL NEOPLASIA OF THE UTERINE CERVIX

N. Wentzensen, C. Bergeron, F. Cas, D. Eschenbach, S. Vinkurova and M. von Knebel Doeberitz

The identification of a small percentage of high-grade cervical intraepithelial neoplasia (HGCIN) among patients with a diagnosis of atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesion (LSIL) is one of the difficulties in cytology-based cervical cancer screening. p16INK4a is a surrogate marker for the initiation of HPV-mediated cervical carcinogenesis. We have used the detection of the protein p16INK4a by immunocytochemistry coupled with a nuclear score to differentiate abnormal cells from metaplastic or atrophic cells. In a pilot series of 210 liquid-based cytology (LBC) specimens of which 108 were considered normal, 52 with a diagnosis of LSIL and 50 with a diagnosis of high-grade SIL (HSIL) the presence of p16 positive cells with a score > 2 showed a good correlation to an HSIL result. In a subsequent series 137 LBC specimens with an ASCUS result and 88 with an LSIL result were included that were all followed by colposcopy and biopsy. The overall sensitivity for the diagnosis of HGCIN considering labeled squamous cells with a nuclear score > 2 was 96% and the specificity was 83%. The sensitivity in the ASCUS was 95% and the specificity was 84% and in the LSIL group 100% and 81%, respectively. These data warrant a study of a large series of LBC smears with a diagnosis of ASCUS and LSIL to confirm its efficacy of predicting the presence of an HGCIN.

From the University of Vermont, Burlington, Vermont, U.S.A.

A-09 P16 AS A REFLEX INVESTIGATION IN HIGH-GRAGE SQUAMOUS INTRAEPITHELIAL LESION TO AVOID UNNECESSARY COLOPSCOPY

M. Harvey, G. Leiman, W. Trotman, J. Sherman and M. Evans

BACKGROUND: Nuclear expression of the protein p16INK4a is seen in cervical squamous epithelium with genome-integrated high-risk human papillomavirus (HPV) subtypes. This study determines if p16INK4a can be used to triage the Bethesda category of atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion (ASC-H) in thin-layer Papanicolaou (Pap) tests, to reduce unnecessary colposcopy.

MATERIALS AND METHODS: Residual material from Thin-Prep vials on ASC-H samples was stained with p16INK4a using the Dako antibody system. Immunostaining was interpreted using published criteria, with grades 0, 1 and 2 considered negative and grades 3 and 4 positive. All cases were then submitted for HPV analysis using the nested polymerase chain reaction (PCR) method. Biopsy results were obtained and were correlated.

RESULTS: A total of 81 cases were tested. After exclusion of 24 cases without follow-up and 2 cases for technical reasons, 73% of ASC-H Pap tests were positive by p16INK4a (n = 40), 51% on histology (n = 30) and 98% positive for high-risk HPV by PCR (n = 54). Correlation of Pap test p16INK4a and PCR with initial colposcopic biopsy was performed to obtained statistical values. p16INK4a demonstrated 93% sensitivity, 68% specificity, 70% positive predictive and 87% negative predictive value. Using the χ² method, p16INK4a results yielded a p value of 0.001. Nested PCR showed 100% sensitivity, 96% specificity, 68% positive predictive value, and 100% negative predictive value with a p value of 0.2%.

CONCLUSION: p16INK4a is highly sensitive for high-risk HPV infections in the setting of ASC-H patients. The negative predictive value of 87% is sufficiently high to triage patients, suggesting routine p16INK4a may prove to be valuable in the management of ASC-H patients. The negative predictive value appears great enough to avoid colposcopy for patients negative by p16INK4a testing. Nested PCR performance is robust but overly sensitive and thus not clinically useful.

From the Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, Germany, and the Laboratory Pasteur-Cerba, Paris, France.

A-10 APPLICATION OF IMMUNODIAGNOSTIC MARKERS GALECTIN-3 AND HBME-1 TO FINE NEEDLE ASPIRATION SMEARS OF THYROID NODES TO IMPROVE DIAGNOSTIC ACCURACY WITH HISTOLOGY

C. Van Wyk, M. Louw, and C. Wright

Fine-needle aspiration cytology (FNAC) is a widely used preoperative procedure to diagnose benign or malignant thyroid nodules. However, the interpretation of follicular lesions in thyroid aspirates have been a diagnostic pitfall for cytopathologists based on the overlapping cytologic features between benign or malignant thyroid follicular lesions. The objective of this study was the application of immunodiagnostic markers galectin-3 and HBME-1 to FNA cytology smears and to determine their sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy for malignancy in thyroid lesions. Furthermore, to establish if these antibodies can facilitate diagnostic accuracy of FNA cytology smears with the histologic diagnosis. Papanicolaou-stained FNA smears of 62 patients who presented with thyroid nodules during June 2001 and July 2005 and their corresponding paraffin-embedded tissue blocks were retrieved from the archives at the Anatomical Pathology Laboratory, NHLS, Tygerberg Hospital, Cape Town. The neoplastic thyroid lesions included 21 carcinomas, 16 follicular and Hürtthle cell adenomas and 25 nonneoplastic lesions. Immunohistochemical staining was carried out with the avidin-biotin-peroxidase complex method using dianimobenzidine (DAB) as substrate. Statistical analysis had shown that the sensitivity, specificity, PPV, NPV and diagnostic accuracy for galectin-3 in malignant tissue sections were 90%, 83%, 73%, 94% and 86% and for HBME-1, 95%, 68%, 61%, 97% and 77%, respectively. The sen-
The 94 FNA specimens for which there was histologic follow-up received at the MUHC Cytopathology laboratory in 2003 and 2004. Immunopositivity for both markers was also significantly more in malignant than benign lesions. However, their ability in distinguishing follicular carcinomas from adenomas was not so significant.

From the Cytology Unit, Department of Pathology, NHLS, Tygerberg, South Africa.

A-11 CYTOLOGIC FINDINGS OF CARCINOMA SHOWING THYMUS-LIKE DIFFERENTIATION (CASTLE) OF THE THYROID

M. Hirokawa, S. Kuma, M. Maekawa and A. Miyachi

BACKGROUND: Carcinoma showing thymus-like differentiation (CASTLE) is a very rare intrathyroidal neoplasm affecting thyroid and neck soft tissues, which has to be distinguished from squamous cell and anaplastic thyroid carcinoma because it has a better prognosis. To the best of our knowledge, so far, 1 case describing the cytologic findings of this tumor has been reported.

Methods: We reviewed 6 CASTLE cases that received aspiration cytology of the thyroid. Four patients were women and 2 were men. The age range was 25–73 years (average 44).

RESULTS: The cellularity was moderate to marked, and lymphocytes were seen in the background. Two cases also presented neutrophils. The tumor cells were round to spindle-shaped and formed variable-sized cell clusters. Squamous cell differentiation was observed in 4 cases and 2 of them showed keratinization. Two cases revealed intracytoplasmic lumina.

CONCLUSION: It should be known that differential diagnosis of CASTLE is not anaplastic carcinoma but squamous cell carcinoma or mucoepidermoid carcinoma, because mucin-producing tumor cells may be apparent. Metastatic carcinoma should be also differentiated.

From the Departments of Diagnostic Pathology and Cytology and Surgery, Kuma Hospital, Kobe, Japan.

A-12 QUANTITATIVE ASSESSMENT OF NUCLEAR GROOVES IN NEOPLASTIC AND NONNEOPLASTIC LESIONS IN FINE NEEDLE ASPIRATION SAMPLES OF THE THYROID: A RETROSPECTIVE CYTOLOGIC/HISTOLOGIC STUDY OF 94 CASES

E. Alkuwari, K. Khetan, N. Dendukuri, L. Wang and M. Auger

OBJECTIVE: The aim of the study was to determine if there is a quantitative cutoff where nuclear grooves can predict the presence of papillary carcinoma vs. other neoplastic and nonneoplastic lesions in fine needle aspiration (FNA) samples of the thyroid. methods: We reviewed all the thyroid FNA cases (N = 1,775) received at the MUHC Cytopathology laboratory in 2003 and 2004. The 94 FNA specimens for which there was histologic follow-up were selected. Each FNA was assessed by 3 pathologists blindly (i.e., with no knowledge of the original cytologic and histologic diagnoses). The percentage of nuclear grooves was determined quantitatively by manual counting of nuclear grooves per 300 cells in areas with most frequent grooves at ×400 in the FNA specimens.

RESULTS: There were 38 (40.4%) cases of papillary thyroid carcinoma (PTC), 24 (25.5%) cases of other thyroid neoplasms and 32 (34.1%) cases of nonneoplastic lesions on final histology. The mean percentage of nuclear grooves was 23.7% in PTC vs. 9.44% in non-PTC cases. Hurthle cell metaplasia in non-PTC cases was associated with higher frequency of grooves (11.3%) than cases without metaplasia (9.8%). The predictive value of the percentage of cells with nuclear grooves for a diagnosis of PTC (at 95% CI) was 0.66 for a 15% cutoff, 0.82 for a 20% cutoff and 0.85 for 25% cutoff. Therefore a value of 20% cells with nuclear grooves appears to be the most useful threshold for predicting PTC. Frequent causes of false negative FNA diagnoses of PTC (<20% nuclear grooves) were borderline cellularity and poor cellular preservation.

CONCLUSION: We concluded that a quantitative counting method of nuclear grooves is useful in evaluating thyroid FNAs in the diagnosis of neoplastic and nonneoplastic thyroid lesions. The presence of ≥20% or more nuclear grooves is highly suggestive of PTC.

From McGill University Health Center, McGill University, Montreal, Quebec, Canada.


B. Smith and P. Thomson

OBJECTIVE: An educational review of diagnostic errors in salivary gland fine needle biopsies (FNBs).

METHODS: Review of in-clinic FNBs from 1995 to 2003. The outcome was retrieved from patient files or from referral letters to the physician. Discrepant cases with the incorrect diagnosis of pleomorphic adenoma (PA) were blindly reviewed.

RESULTS: There was a total of 129 FNBs with a diagnosis of PA. Of these, 59 FNBs had histologic follow-up and 51 (86%) had a correct diagnosis. Of the 8 errors, 4 were benign (1 benign salivary gland tissue, 1 basal cell adenoma, 2 myoepitheliomas) and 4 were malignant (2 adenoid cystic carcinomas, 1 acinic cell carcinoma, 1 mucoepidermoid carcinoma). FNB material was not available for review in 1 case (adenoid cystic carcinoma). Each of the 7 reviewed FNBs were not quite typical for PA. Errors included interpreting scant myxoid material in benign salivary gland tissue as PA (1), interpreting fibrillary stroma in an adenoid cystic carcinoma as defining a PA (1), interpreting bare nuclei in an adenoid cystic carcinoma as myoepithelial cells (1), interpreting myoocytes in a mucoepidermoid carcinoma as myoepithelial cells (1), failing to recognize a monomorphous cell population (myoepithelioma, basal cell adenoma, acinic cell carcinoma), and failing to appreciate cytoplasmic granules in an acinic cell carcinoma (1).

CONCLUSION: The diagnostic accuracy of FNB for PA is high, reflecting the characteristic cytologic features and high pretest probability of disease. Errors were usually interpretive. In FNBs that are suggestive but not typical of PA, it is best to acknowledge diagnostic uncertainty in the report.

From the Cytology laboratory, British Columbia Cancer Agency, Vancouver,
A-14 SPUTUM CYTOLOGY IN ORDER TO DETECT OCCULT PERIPHERAL SQUAMOUS CELL CARCINOMA

M. Shiba and A. Taguchi

OBJECTIVE: Sputum cytology has been proved to be effective to detect central pulmonary squamous cell carcinomas (SCC), but has not been proved to be effective to detect peripheral SCC. We analyzed its efficacy to detect peripheral pulmonary SCC in this series.

METHODS: In Chiba Cancer Screening Center, a total of 91,945 participants in the high-risk group for lung carcinomas were involved in our study between 1993 and 2001. Sputum cytology was performed, and chest x-ray examination, including computed tomography (CT) and bronchoscopy, were added as detailed examinations. We analyzed cytologic findings of peripheral SCC detected and compared with that of central SCC and their chest x-ray.

RESULTS: During this period, 144 lung cancers were found by this program (total detection rate 0.16%). Among them, 21 cases (14.6%) were peripheral SCCs. They were all men and heavy smokers. Clinical stages were 14 cases in stage I, 7 cases in more than stage II, and severe lung emphysemas were combined in 5 cases. At the time of sputum examination, positive plain chest x-ray findings were observed in 9 cases. In 3 cases, positive plain chest x-ray findings were obtained during the follow-up period. In 9 cases (43%), only sputum cytology was positive or suspicious at the time of sputum examination, and bronchoscopy or chest CT showed negative results. In these cases, lesions were detected by chest CT follow-up examination. Compared with central SCCs, numbers of atypical squamous cells appeared in the sputum of peripheral SCCs were few and incoherent. And bright yellow keratinizing atypical squamous cells were dominant.

CONCLUSION: Nine cases of 144 detected lung cancers were so-called occult peripheral SCCs that were negative by bronchoscopy or chest CT examination. Analysis of atypical squamous cells in the sputum of the SCC patients was effective to diagnose the sites of lesions.

From Kmitusu-Chuo General Hospital, Kisarazu; and Chiba Foundation for Health Promotion and Disease Prevention, Chiba, Japan.

A-16 COMPARISON OF CYTOLOGY TEST IN PATIENTS UNDER SURVEILLANCE AFTER TRANSURETHRAL RESECTION OF SUPERFICIAL UROTHELIAL CARCINOMA

M.D. Beccati, C. Buriani, M. Pedriali, P. Querzoli and L. Nencioni

OBJECTIVE: To determine the diagnostic sensitivity of UroVysion test used in a routine clinical practice setting for surveillance of patients in follow-up for superficial urothelial carcinoma. Since bladder cancer has a significant risk of recurrence and progression to invasive disease, the use of sensitive surveillance testing is essential.

MATERIALS AND METHODS: A total of 61 patients (mean age 71.6 years, range 42–91) were evaluated; 53 (87%) had a history of bladder cancer and 8 (13%) presented symptoms. The urine specimens (57 voided urine and 8 renal washes) were evaluated by liquid-based cytology (ThinPrep) and UroVysion FISH. After conventional cytology, FISH was performed from the residual material (reflex FISH). Multicolor FISH includes centromere-specific probes for chromosomes 3, 7, 17 and a locus-specific probe for 9p21/p16. Twenty-six patients (43%) also underwent biopsy. We compare cytologic diagnosis with UroVysion test results and histologic diagnosis, when available.

RESULTS: It was possible to evaluate the UroVysion test in 57 (88%) of the 65 specimens, with 18 negatives (28%) and 39 positives (60%). FISH and cytology were concordant in 81% of cases (23% negatives and 58% positives). For the discordant cases, cytology was negative and FISH positive in 10%, and cytology was positive with a negative FISH for 9%. Using concurrent biopsy as the gold standard, the sensitivity and the negative predictive value of UroVysion test are both of 100%. The sensitivity of cytology is 88%, and the negative predictive value is 67%.

CONCLUSION: FISH is significantly more sensitive than cytology in diagnosing urothelial carcinoma. Given the high negative predictive value of the UroVysion test, using both cytology and UroVysion tests in surveillance protocols for patients with superficial urothelial carcinoma may reduce the number of cystoscopies, performing cystoscopy only on patients with positive FISH tests.

From the Departments of Diagnostic Cytopathology S. Anna University Hospital; and Department of Surgical Pathology, University of Ferrara, Ferrara, Italy.
A-17 PATTERNS OF METASTASIS TO THE SUPRACLAVICULAR LYMPH NODES

G. A. Barkan, P. Gattuso, M. McIntyre, S. Bakhos, P. Pioro, E. M. and Wojcik

BACKGROUND: SuprACLAVICULAR lymph nodes (SCLN) have been called sentinel nodes because of their accessibility and their affinity for metastases. Diagnosis rendered by fine needle aspiration (FNA) of SCLN can provide staging and prognostic information. The aim of this study is to identify patterns of metastases to the SCLN.

METHODS: An electronic search was undertaken from 1990 to 2006 in the Loyola University Medical Center and the Maywood Rush University Medical Center, Chicago. A total of 218 cases of FNA of SCLN were identified. Medical records were reviewed and age, sex, site, FNA diagnosis and previous medical history were recorded.

RESULTS: A total of 218 SCLN FNAs constituted the study group (111 women [mean age 60] and 107 men [mean age 63]). Of these 149 of 218 (68.4%) aspirates were on the left and 69 of 218 (31.6%) were on the right side. Malignant aspirates constituted 188 of 218 (86.2%) of the cases (102 adenocarcinoma, 33 squamous cell carcinoma (SCCA), 8 small cell carcinoma (SMCCA), 31 lymphoma and 14 miscellaneous malignancies). The most common malignancies were SCCA (33), breast adenocarcinoma (31), and lymphoma (31). Metastases were located mostly on the left 131 of 147 (89.1%) and rarely 14 of 147 (10.9%) on the right side. Adenocarcinoma metastases 77 of 102 (75.5%) were mostly found on the left side. Similarly, the lymphoma 22 of 31 (70.9%), SMCCA 6 of 8 (75%) and miscellaneous malignancies 9 of 14 (64.2%) had a tendency to the left side. Interestingly, SCCA was found on equally both sides (17 of 33 [51.3%] right; 16 of 33 [48.0%] left). Reactive lymph nodes constituted 25 of 218 (11.3%) of the cases and they were identified mostly on the left side 16 of 24 (66.7%).

CONCLUSION: Majority of FNA samples (86.2%) were metastases, most common being SCCA and breast adenocarcinoma. Both metastases and reactive lymph nodes tended to be localized on the left, except for SCCA. This predilection for the left SCLN is probably a result of the lymphatic flow being more prominent on the left side.

From Loyola University Medical Center, Maywood, and Rush University Medical Center, Chicago, Illinois, U.S.A.

A-18 A NEW METHOD OF BILIARY TISSUE SAMPLING FOR CYTOPATHOLOGIC EXAMINATION DURING ENDOSCOPIC RETROGRADE CHOLANGIOGRAPHY


OBJECTIVE: Biliary brushing during endoscopic retrograde cholangiography (ERC) may allow diagnosis of cancer, but this technique is limited by a low (18–57%) sensitivity. We compared the cellular and diagnostic yields of a new sampling method (using structure dilation and a grasping basket) to those of brushing.

METHODS: Sixty consecutive patients with a suspected malignant obstruction of the common bile duct were included in the study in a consecutive, nonrandomized, order. They underwent sampling during ERC using the new method (study patients, n = 30) or a brush (control patients, n = 30). A final diagnosis of cancer was made in 22 of 30 (73%) study patients and 21 of 30 (70%) control patients. At the end of the study period, all smears were interpreted at cytopathologic examination in a blind and random fashion for cellularity (insufficient, low, moderate, or high) and diagnosis (normal, atypical considered reactive, highly atypical suspicious for cancer, or malignant). “Benign” samples showed cells arranged in coherent plaques, round or oval nuclei and inconspicuous nucleoli. “Atypical benign” cases displayed coherent crowded cells with nuclear hyperchromasia and prominent nucleoli. “Atypical malignant” cases displayed enlarged cells with vesicular nuclei and conspicuous nucleoli. “Malignant” cases were both enlarged and crowded with prominent nucleoli and isolated cells were present. “Highly atypical” and “malignant” diagnoses were considered as indicative of cancer.

RESULTS: Compared to biliary brushing, the new method provided a high cellular yield in more cases (19 of 30 vs. 10 of 30; p = 0.029), a higher sensitivity for the detection of cancer (19 of 22 [86%] vs. 11 of 21 [52%]; p = 0.015), and a higher diagnostic accuracy (27 of 30 [90%] vs. 20 of 30 [67%]; p = 0.028). Specificity for the detection of cancer was 100%. The 30-day complication rate observed with the new method was 3%.

CONCLUSION: Significantly higher cellular and diagnostic yields can be obtained at ERC in patients with a suspected malignant biliary stricture by using this method compared to brushing.

From Hospital Petronian and Hospital Italiano, Buenos Aires, Argentina; and Geneva University Hospital, Geneva, Switzerland.

A-19 AN AUTOMATED NANOLITER DISPENSER FOR STAINING INDIVIDUAL BIOPSIES IN TISSUE MICROARRAYS


The development of tissue microarrays (TMAs) significantly improved the efficiency of immunohistochemical staining by providing a platform in which hundreds of biopsies could be stained simultaneously on a single slide. Ideally, the functionality of TMAs will be extended to performing multiple tests on each TMA slide. We present the tissue microarray antibody spotter (TMAS) being developed at the University of British Columbia (UBC) Applied Biophysics Laboratory to automate immunocytochemical staining of biopsies within a TMA on a biopsy-by-biopsy basis, as opposed to the conventional method of applying a single reagent to an entire TMA slide. This technology enables each biopsy or combination of biopsies to be treated with a different reagent, allowing multiple tests to be performed in parallel, and the ability to provide a complete pathologic profile from a single TMA slide. Furthermore, by delivering reagents directly to biopsies, without coating the glass substrate between biopsies, significant cost savings are achieved. Reagent use scales with the number of biopsies being tested, result-
ment of an entire TMA slide with a single reagent. Run-to-run cross-contamination and diffusion between biopsies are undetectable. A beta-prototype is currently in development for testing and evaluation at collaborator sites in clinical and research environments. The ultimate applications of the TMAS will be to interrogate multiple biomarkers on cytology arrays assembled from a single fine needle aspiration biopsy and to enable rapid titration of reagents when evaluating new antibodies or probes against cancer biomarkers. This work is funded by Genome British Columbia.

From The University of British Columbia Applied Biophysics Laboratory; Genome Sciences Centre, British Columbia Cancer Agency; and James Hogg iCapture Centre, Vancouver, British Columbia, Canada.

A-20 CALCULATING COST BY INTRODUCING AUTOMATION IN THE CYTOLOGY LABORATORY

B. Bjerregaard and J. Kjelleberg

OBJECTIVE: Several studies have been published discussing the clinical effectiveness of liquid-based cytology (LBC) and computer-assisted diagnosis (CAD) in cervical cytology. Very few studies have been published discussing the costs by introducing these techniques. The aim of this study was to calculate cost of LBC or conventional smear techniques (CST) alone and both in combination with CAD. The results were part of a Danish HTA report (2005).

MATERIALS AND METHODS: A time study using the so-called continuous method was performed in 4 Danish laboratories. 2 using LBC and 2 using CST. The time for all activities for cytotechnologists and pathologist was measured from receiving the specimen to a final diagnosis. Costs for utensils were based on information from the Companies.

RESULTS:

<table>
<thead>
<tr>
<th></th>
<th>TriPath LBC</th>
<th>Cytyc LBC</th>
<th>CST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registration cytotechnicians</td>
<td>12.7 (2.3 min)</td>
<td>17.2 (1.7 min)</td>
<td>17.7 (1.4 min)</td>
</tr>
<tr>
<td>Preparation cytotechnicians</td>
<td>8.3 (1.5 min)</td>
<td>1.7 (0.1 min)</td>
<td>2.8 (0.5 min)</td>
</tr>
<tr>
<td>Microscopy cytotechnicians</td>
<td>24.2 (4.4 min)</td>
<td>40.2 (3.1 min)</td>
<td>73.4 (13.4 min)</td>
</tr>
<tr>
<td>Pathologist</td>
<td>5.0 (0.5 min)</td>
<td>2.5 (0.5 min)</td>
<td>25 (5.1 min)</td>
</tr>
<tr>
<td>Quality assurance</td>
<td>5.5 (1.4 min)</td>
<td>5.5 (1.4 min)</td>
<td>55 (11.5 min)</td>
</tr>
<tr>
<td>Utensils incl. container for HPV</td>
<td>38.0</td>
<td>51.7</td>
<td>55 (11.5 min)</td>
</tr>
<tr>
<td>Total</td>
<td>92.7</td>
<td>108.3</td>
<td>101.6</td>
</tr>
</tbody>
</table>

*Excluding sick leave, holiday, lunch breaks, meetings and administrative tasks.

Cost in Danish Kroner (and Minutes)* of LBC and CST

<table>
<thead>
<tr>
<th></th>
<th>TriPath LBC</th>
<th>Cytyc LBC</th>
<th>CST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost except microscopy cytycotechnicians</td>
<td>68.5</td>
<td>68.1</td>
<td>28.2</td>
</tr>
<tr>
<td>Computer-assisted microscopy, including</td>
<td>14.6</td>
<td>14.6</td>
<td>17.0</td>
</tr>
<tr>
<td>Loading and unloading</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Guided microscopy (50% TriPath),</td>
<td>6.6</td>
<td>10.0</td>
<td>6.6</td>
</tr>
<tr>
<td>50% Cytyc)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leasing and support-guided microscopy</td>
<td>6.1</td>
<td>10.0</td>
<td>18.4</td>
</tr>
<tr>
<td>Microscopy cytotechnicians (25%)</td>
<td>98.0</td>
<td>115.6</td>
<td>72.4</td>
</tr>
</tbody>
</table>

CONCLUSION: When introducing computer-assisted diagnosis in the cytology laboratory, the conventional smear technique is the most cost effective, including extra containers for examination for human papillomavirus.

From the Department of Pathology, Herlev Hospital, Herlev; and Danish Institute for Health Services Research, Copenhagen Denmark.

A-21 THINPREP IMAGING SYSTEM EFFECTIVENESS IN THE DETECTION OF GLANDULAR ABNORMALITIES

M. Friedlander, D. Rudomina, R. Gatscha and O. Lin

OBJECTIVE: The ThinPrep Imaging System (TIS) is an FDA-approved tool used to decrease the number of false negative specimens in ThinPrep (TP) gynecologic specimens. Although the increased detection of squamous abnormalities using the TIS has been well documented, data on the impact of the TIS in the detection of glandular abnormalities is limited. The goal of this study is to evaluate the effectiveness of the TIS in detecting glandular abnormalities in cervicovaginal specimens.

METHODS: TP gynecologic specimens evaluated using the TIS and with histologic confirmation of adenocarcinoma involving the gynecologic system were included in this study. Two cytotechnologists independently reviewed the cases unaware of the initial cyto logic diagnosis (primary review) but aware that all patients had histologically confirmed glandular disease on follow-up. Data collected included presence or absence of atypical glandular cells within and outside of 22 fields of view (FOV). Secondary review results were correlated with initial cyto logic and histologic diagnoses.

RESULTS: One hundred twenty-four cases met the criteria for inclusion in this study. Seventy of 124 (56%) cases contained malignant glandular cells in the TP slide. Ninety-seven percent (68 of 70) of these cases contained atypical cells in the 22 FOV. TIS failed to identify atypical glandular cells in 3 cases, which were noted on the slide during secondary review. In the discrepant cases, initially reported as negative for intraepithelial lesion or malignancy, were found to contain atypical glandular cells upon secondary review. All but one of these cases contained atypical glandular cells in the 22 FOV. Most of these atypical cases (6 of 9) derived from endometrial adenocarcinoma of endometrioid type. No cytologic evidence of a glandular abnormality was found in the 54 remaining cases.

CONCLUSION: The TIS was effective in identifying atypical glandular cells in specimens containing malignant glandular cells, leading to a full review of the slide.

From the Memorial Sloan-Kettering Cancer Center, New York, New York, U.S.A.

A-22 APPLICATION OF DIGITAL SLIDE SCANNING TO CYTOLOGIC DIAGNOSIS


Some digital slide systems have already been put to practical use. The NanoZoomer (Hamamatsu Photonics, Japan) is a tissue glass slide–scanning system, which was developed for digital pathology, and converts slides to 1.9-gigapixel high-resolution digital images in approximately 3 minutes per slide, scanning a 20×20 mm field of a glass slide in standard mode (≥20 mode). We attempted to adapt this digital slide system for cytologic specimens. When cytologic specimens are scanned, higher resolution images are required. Images of 7.6 gigapixels (×40 mode) are suitable for cytologic specimens. Digital slide technology for cytology will realize cytoscreening without microscopy. Observation using a PC monitor will enable more accurate cytoscreening without overlooking any part of the cytologic
specimen. Furthermore, rapid cytologic diagnosis at hospitals with neither resident cytoscreeners nor cytologists can easily be realized by the establishment of a telecytology network. In addition, by establishing a network among cytologists it is possible to consult about difficult cases with remote specialized cytologists. Another benefit of digital slides is slide storage. A large space is needed for glass slide storage. Digital data derived from 800 glass slides can be stored on a 500-gigabyte hard disk, which creates a big advantage in terms of space saved for slide storage. Furthermore, the system has the potential to reduce concerns about damage, deterioration or loss of cytologic specimens, which are common with glass slides. However, one problem remains to be resolved. Cytologic samples often have some thickness caused by the overlapping of cells and cellular clusters. We usually observe cellular clusters with overlapping by changing the focus depth of the microscope. At present, the NanoZoomer has no function that corresponds with changing the focus depth of the microscope. Hamamatsu Photonics is currently developing 3-D scanning for cytologic specimens.

From Tokyo Medical University Hospital, Tokyo, Japan.

DIAGNOSTIC CYTOLOGY: CERVICAL CYTOLOGY QUALITY ASSURANCE LIQUID CYTOLOGY

A-23 CHARACTERISTICS OF FALSE NEGATIVE LIQUID-BASED CYTOLOGY PREPARATIONS

D. Johns, N. Dudding and J. Smith

OBJECTIVE: Although the characteristics of false negative conventional smears have been well documented, there is limited literature available on liquid-based cytology (LBC), especially with regard to SurePath samples.

METHODS: We will present the results of a 16-month audit and describe the typical characteristics of false negative cases in a medium-sized screening laboratory processing approximately 42,000 samples per annum. Cases were identified either from routine rapid screening or by review of negative samples from women with a known cervical intraepithelial neoplasia (CIN) lesion.

RESULTS: A total of 100 cases were identified: 156 by rapid screening, the remainder by review of false negative samples from women with known CIN. Of those identified by rapid screening, 127 were reported as borderline nuclear change, 12 as mild, 4 as moderate, 9 as severe and 4 as glandular neoplasia. Only those cases that were found to have a histologic lesion were used in the study (n = 36): 32 were identified by rapid review and 4 by review of previous negative samples. Eight were found to have CIN 1, 10 CIN 2, and 16 CIN 3. One was identified as CGIN and 1 as endometrial adenocarcinoma.

CONCLUSION: Examples of the typical characteristics will be described. Features similar to those observed in conventional smears were identified; scanty abnormal cells (< 30 per preparation), low-contrast samples, pale cells and microbodies. In contrast to conventional smears, missed abnormal cells were almost uniformly scattered throughout the sample.

From Sheffield Teaching Hospitals, Sheffield; and East Pennine Cytology Training Centre, Leeds, U.K.

A-24 A 10-YEAR REVIEW OF GLANDULAR CYTOLOGY REPORTS WITH SPECIAL REGARD TO THE EFFECT OF CONVERSION TO LIQUID-BASED CYTOLOGY

J. Smith, E. Shore, A. Ash and N. Dudding

Recent reviews of glandular reports have confirmed a wide variation in specificity.1,2 We have reviewed our performance over the last 10 years and evaluated the effect of conversion to liquid-based cytology (LBC) on our reporting rates and accuracy. The review revealed an upward trend in the ability to accurately detect glandular lesions, with particular improvement in identification of cervical glandular intraepithelial neoplasia (CGIN). The positive predictive value (PPV) for a report of glandular neoplasia as per U.K. cervical screening reporting guidance rose from 51% in 1996 to 93% in 2005 for any neoplastic lesion and from 21% in 1996 to 83% in 2005 for a correctly identified glandular neoplastic lesion. Reasons for this general increase will be discussed, including changes to the laboratory staffing structure and better recognition of such lesions through improved training. In addition, the recent introduction of LBC technology may have resulted in improved sampling of the endocervical component through use of the Cervex Broom and smear taker training and SurePath cell collection techniques with enhanced preservation of morphology may have led to better recognition.

References


From Royal Hallamshire Hospital, Sheffield, U.K.

A-25 SAMPLING ADEQUACY OF FALSE NEGATIVE LIQUID-BASED CERVICAL CYTOLOGY SPECIMENS IN TISSUE-PROVEN MODERATE TO SEVERE CERVICAL DYSPLASIA

W. Lachar and P. Valente

Efforts have been made to develop adequacy criteria for liquid-based cervical cytology (LBC) specimens to minimize the number of false negative diagnoses, with a requirement of at least 5,000 squamous cells on a ThinPrep slide. This study was undertaken to compare the squamous and endocervical cellularity of false negative and true positive LBC specimens in patients with a proven tissue biopsy of cervical intraepithelial neoplasia (CIN) grades II and III. LBC specimens from 24 patients with a subsequent tissue biopsy diagnosis of CIN II or III were reviewed. Twelve were negative for dysplasia or were given a diagnosis of atypical squamous cells of undetermined significance (ASCUS), and 12 were diagnosed as high-grade squamous intraepithelial lesion (HSIL). Each ThinPrep slide was divided into 4 quadrants, the number of squamous cells per high power field (×400) was counted in 5 fields per quadrant, and a total number of squamous cells per slide was extrapolated. The total numbers of groups of endocervical cells and dysplastic cells on the
entire slide were also counted. There was no significant difference in the number of squamous or endocervical cells among the false negative and true positive specimens (p = 0.09 and p = 0.06, respectively). The extrapolated number of squamous cells ranged from 2,018 to 42,499, and total endocervical cell groups ranged from 0 to 142. Contrary to expectations, the mean number of endocervical cells in the true positive cases was less than in the false negative cases (averaging 21.4 and 33.8, respectively). The average number of dysplastic cells among the true positive cases was 139, with a range of 17–472. A lack of significant difference of squamous and endocervical cellularity for false negative and true positive LBC specimens does not support a specific cell number as a criterion for specimen adequacy.

From the University of Texas Health Science Center, San Antonio, Texas, U.S.A.

A-26 DETERMINING THE SUPERIORITY OF A NEW TEST: LIQUID-BASED CYTOLOGY VS. CONVENTIONAL SMEAR TECHNIQUE

B. Bjerregaard and M. Lidang

OBJECTIVE: Several publications show that liquid-based cytology (LBC) increases the number of changes, especially low-grade changes (LSIL), compared to conventional smear technique (CST). HTA reports and reviews show conflicting results concerning the clinical effectiveness of LBC compared to CST. It is pointed out that the controversy is due to lack of high-quality studies. The aim of this study was to review publications comparing LBC and CST and evaluate the study design. Part of the study has been published in a Danish HTA report (2005).

METHODS: We searched relevant databases from 1996 to September 2006. Criteria for inclusion were (1) LBC vs. CST, (2) FDA-approved LBC, (3) screening population, (4) Bethesda classification, (5) verification of at least 10% positive and 10% negative cytology.

RESULTS: We found 66 articles comparing LBC with CST. Six articles were excluded for various reasons (other LBC, selected population, and lack of verification). Six articles fulfilled the criteria. In 2 articles there was blinded verification by colposcopy or histology of all diagnoses.1,2 When using LBC instead of CST, both showed a lower sensitivity for LSIL, and Taylor2 also showed lower sensitivity for HSIL. In 4 articles, all using cytopathic verification of negative cytology, the figures did not allow for reliable calculation of sensitivity and specificity.

CONCLUSION: The results of our study showed that there is no scientific basis to suggest any difference in clinical effectiveness between LBC and CST. The only 2 studies with a sufficient study design showed that CST detected more true positive cases than LBC. Several studies recommend laboratories to convert to LBC, but there is a lack of high-quality studies proving that the more expensive new LBC is better that the old CST.

References

A-27 INCIDENCE OF CERVICAL CANCER IN WOMEN AROUND AGE 50 WITH A HISTORY OF 3 OR MORE NEGATIVE SMEARS

M. Rebolj, M. van Ballegooijen, L. Essink-Bot, R. Boer and D. Habbema

OBJECTIVE: The balance between prevented cancer mortality and the screening-induced morbidity and anxiety should be monitored closely. It has been previously suggested that cervical cancer screening could be ceased for women who have accumulated negative smears by age 50, as the occurrence of cervical cancer is lower than among younger women with a similar screening history. We analyzed whether a similar risk pattern could be observed in the Netherlands in the recent period.

METHODS: Information on all cervical examinations in the Netherlands was retrieved from the nationwide registry of histopathology and cytopathology (PALGA). Women with at least 3 negative smears after 1993 were followed until the diagnosis of cervical cancer or until the end of 2002. Women were censored at the first nonnegative smear or at a biopsy. Age at each negative smear was grouped into the following age-groups: < 45, 45–54, and > 54. Cumulative cancer incidence rates were compared by age-group.

RESULTS: After the third or later consecutive negative smear, 61, 34 and 19 invasive cancers were found within 5 years in 740,000, 470,000 and 210,000 women aged < 45, 45–54, and > 54, respectively. Five-year cumulative cancer incidence reached the level of 2 per 10,000 women in women aged 45–54 and was not significantly different from that in other age-groups.

CONCLUSION: Our preliminary results suggest that in the Netherlands women with a multiple negative screening history by age 50 still are at risk for invasive cervical cancer, which is similar to that of younger women. A possible explanation is that women around age 50 can still acquire new human papillomavirus (HPV) infections. All women should therefore remain in the regular screening program until they turn 60.

From Erasmus MC, Department of Public Health, University Medical Center, Rotterdam, the Netherlands.

This study was financed by the National Health Insurance Council (College voor Zorgverzekeringen, grant number 530/003/2002).

A-28 EVALUATION AND MANAGEMENT OF CERVICAL HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION IN ADOLESCENT AND YOUNG WOMEN

C. Zhou, M. Hayes, D. van Niekervk and V. Moravan

OBJECTIVE: To evaluate the risk of cervical high-grade squamous intraepithelial lesion (HSIL) and its management in adolescent and young women in a population-based cervical cytology screening program.

METHODS: Women ≤ 20 years with abnormal cervical cytology between 1997 and 2003 were identified from the database of the cervical cytology screening program of the Province of British Columbia, Canada. Follow-up histology and management records within 3 years of abnormal cervical cytology were also collected from the database.

RESULTS: A group of 21,517 young women with abnormal cervical cytology were identified from a total of 276,565 Pap smears between 1997 and 2003. The detection rate of HSIL cytology among the screened young women was increasing with age: age 11–15,
A-29 CYTOLOGY AND OUTCOME OF LOW-GRADe SQUAMOUS INTERTHEPHELIAL LESIONS WITH OCCASIONAL CELLS SUSPICIOUS FOR HIGH-GRADe SQUAMOUS INTERTHEPHELIAL LESIONS COMPARED TO HIGH-GRADe ATYPICAL SQUAMOUS CELLS

M. Duggan, P. Power, J. Gregoire and J. Nation

OBJECTIVE: Cytology and clinical outcomes associated with Pap tests reported as high-grade atypical squamous cells (ASC-H) and as “low-grade squamous intraepithelial lesion (LSIL) containing occasional cells suspicious for high-grade squamous intraepithelial lesion (HSIL) (LSIL-H)” were compared. In much of Canada, women with ASC-H are referred for colposcopy and women with LSIL-H is not included in the Bethesda System and is characterized in terms of cytologic features and clinical course.

METHODS: All ASC-H (n = 153) and LSIL-H (n = 189) Pap tests recorded in the regional laboratory information system in 2001 were identified. All available histology results for each associated patient over the subsequent 2-year period were retrieved to determine clinical outcome. Pap tests were reviewed to characterize cytologic features.

RESULTS: An HSIL+ (CIN II or III or carcinoma) was identified in 48% of the ASC-H and 40% of the LSIL-H group (p = 0.136). Cytologic features included a single HSIL cell, 1 or more cells with a markedly increased nucleus to cytoplasm ratio whose evaluation was limited by artifact, parakeratotic cells with enlarged irregular nuclei and enlarged squamous cells with giant or multiple nuclei. The LSIL-H test additionally showed koilocytes.

CONCLUSION: The cytologic features of cells suspicious for HSIL were similar in Pap tests reported as ASC-H and LSIL-H. Since both groups predicted an HSIL+ outcome with a somewhat high frequency and similar accuracy, both should be managed by immediate referral to colposcopy.

From the University of Calgary, Calgary, Alberta; Memorial University, St. John’s, Newfoundland; and Centre Hospitalier Universitaire de Quebec, Quebec, Canada.

A-30 WOMEN WITH CERVICAL INTRAEPITHELIAL NEOPLASIA AT INCREASED RISK FOR INVASIVE CERVICAL CANCER AFTER COMPLETING RECOMMENDED FOLLOW-UP

M. Rebolj, M. van Ballegooijen, L. Essink-Bot, R. Boer and D. Habbema

OBJECTIVE: Women with a preinvasive cervical lesion (cervical intraepithelial neoplasia [CIN]) are advised to rejoin the regular 5-year screening program after they had 3 consecutive posttreatment negative Pap smears. We evaluated their 5-year incidence of subsequent invasive cervical cancer.

METHODS: Information on all cervix uteri examinations in the Netherlands was retrieved from the nationwide registry of histopathology and cytopathology (PALGA). We identified women with the first CIN 1–3 diagnosis and 3 subsequent negative smears who had no previous cytologic or histologic abnormality. Women were censored at the cancer diagnosis, or if they had any subsequent abnormality. For the period 1994–2002, the age-adjusted cumulative 5-year incidence of invasive cancer for these women was compared to that of women with completely negative screening histories.

RESULTS: We identified almost 20,000 women with a satisfactorily completed follow-up after the first CIN diagnosis, 19 of these developed cervical cancer. Among 1,24 million women with negative smears, 356 developed cervical cancer. The age-adjusted 5-year cumulative incidence of cancer in women with a CIN diagnosis is 1.4%, whereas in women with a negative screening history it is 0.2% per 10,000 women. This difference is statistically significant (p < 0.001).

CONCLUSION: These preliminary results suggest that the 5-year cumulative incidence of invasive cancer in women with the first CIN episode and a satisfactory follow-up is high compared to other women in the regular screening program. A tentative explanation for this finding could be a variation in individual risk for cervical cancer. However, cost-effectiveness of different instruments to decrease the incidence should also be investigated, for example, substituting the Pap smear in posttreatment follow-up by testing for HPV, prolonging the post-treatment follow-up or adjusting the screening scheme according to the woman’s screening history.

From Erasmus MC, Department of Public Health, University Medical Center, Rotterdam, the Netherlands.

This study was financed by the National Health Insurance Council (College voor Zorgverzekeringen, grant number 530/003/2002).

A-31 PROPOSED NEW ADEQUACY CRITERIA FOR A PAP TEST IN POSTMENOPAUSAL PATIENTS


BACKGROUND: The current Bethesda System (2001) for reporting cervical cytology includes 2 categories of adequacy: satisfactory and unsatisfactory for every woman regardless of the menopausal status. For the liquid-based preparations an estimated minimum of at least 5,000 well-visualized and well-preserved squamous cells are required for the specimen to be satisfactory. Given the natural history of atrophy in postmenopausal patients, the current criteria for evaluation of adequacy maybe too stringent.

STUDY DESIGN: Between the years 2001 and 2006 there were 510 unsatisfactory ThinPrep Pap tests in women > 45 years diag-
**A-32 APPLICATION OF LIQUID-BASED PREPARATION ON FINE NEEDLE ASPIRATION CYTOLOGY OF BREAST CANCER**


**BACKGROUND:** Liquid-based cytology (LBC) is a well-known procedure used in gynecologic specimens. LBC is also available for nongynecologic specimens, such as fine needle aspirations (FNA). In this presentation, we report the usefulness of LBC for conventional cytologic evaluation, as well as molecular analysis and immunocytochemistry in the cytologic diagnosis of breast cancer.

**METHODS:** Thin-layer cytology preparations were made from the fresh cut-surface of the breast tumors by scraping at intraoperative rapid diagnosis. Comparison of the cytomorphology was made between the routinely processed FNA cytology and the LBC cytology. Immunocytochemical assessment of estrogen receptor (ER), progesterone receptor (PgR) and HER-2 was performed on the LBC preparations. In addition, molecular analysis for P53, ER, PgR and HER-2 was carried out.

**RESULTS:** Generally the cytotologic features of LBC preparations tended to show shrink cell contour and more cytoplasmic cyanophilia in comparison with those of routinely processed FNA cytology. According to the term of the LBC specimens kept in the preservative solution, the nuclear chromatin pattern was getting to be clear with granularity. Both the immunocytochemistry and molecular analysis showed satisfactory results. A good correlation was obtained in the results of HER-2 immunocytochemistry and expression of HER-2 mRNA.

**CONCLUSION:** LBC preparation is a suitable method not only for gynecologic specimens but also nongynecologic FNA specimens.

From Loyola University Medical Center, Maywood, Illinois, U.S.A.

**A-33 TELOMERASE ACTIVITY DEMONSTRATED WITH TELEOMERE REPEAT AMPLIFICATION PROTOCOL IN SITU IN FINE NEEDLE ASPIRATION SPECIMENS FROM FRESH BREAST CANCER TISSUE: A PILOT STUDY**

J. Rehnberg, N. Zendehrokh and A. Dejneka

**BACKGROUND:** Telomerase is up-regulated in over 80% of malignant tumors, making telomerase activity a potential marker for malignancy. With telomere repeat amplification protocol (TRAP) in situ we have previously demonstrated telomerase activity on the cellular level in effusions. Malignant cells usually showed strong activity, and strong activity in stromal cells indicated malignancy. Fine needle aspiration (FNA) is important in the diagnosis of breast tumors, but the differentiation between carcinoma and atypical hyperplasia can be difficult. To examine whether the material obtained in FNA from breast cancers is sufficient for TRAP in situ and if the sensitivity for malignancy and the intensity of the activity makes TRAP in situ of potential use in breast cancer diagnosis.

**MATERIALS AND METHODS:** TRAP in situ was performed on 10 FNA samples from fresh surgical specimens from breast tumors (2 lobular, 8 ductal carcinomas). The intensity of the enzyme activity was assigned to groups (weak [1], moderate [2] and strong [3]). One Giemsa-stained slide and slides for immunocytochemistry (EMA, Cam 5.2, BerEp 4) were made from each specimen, and the cell content was compared with that seen on the TRAP slides.

**RESULTS:** All 10 FNAs tested contained malignant cells. The cell contents of the TRAP in situ and the routine stained slides were similar. All cases were telomerase positive, and activity was seen in the whole malignant population. In 9 of 10 cases the intensity was classified as 2-3. One lobular carcinoma showed weak intensity.

**CONCLUSION:** The cell material obtained in FNA was suitable for demonstrating telomerase activity using TRAP in situ. The sensitivity was 100%, the enzyme activity was usually moderate or strong and seen in all malignant cells in the preparations. The results have to be verified in a larger series, and the specificity has to be addressed by including aspirates from benign breast lesions.

From The Department of Laboratory Medicine, Section of Pathology, Malmo, Lund University, Sweden.

**A-34 DIAGNOSIS OF BREAST CARCINOMA BY ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION AND BY SIMULTANEOUS BREAST CORE BIOPSY: A REVIEW OF 680 CASES**

C. Zhou, T. Thomson and P. Switzer

**OBJECTIVE:** To evaluate the value of ultrasound-guided fine needle aspiration (FNA) compared to simultaneous breast core biopsy (CB) in diagnosis of radiologically detected nonbenign breast lesions.
A total of 680 cases of breast CB with immediate breast FNA were performed for radiologically detected indeterminate, suspicious or malignant lesions from February 2001 to April 2006 in a large urban radiology clinic by radiologists with experiences in ultrasound-guided breast CB and FNA.

RESULTS: FNA diagnosis for the 680 specimens were carcinoma, 433 (63.7%); suspicious, 82 (12.1%); atypia, 61 (8.9%); benign, 46 (6.8%); fibroadenoma, 23 (3.4%); papillary lesion, 10 (1.4%); and nondiagnostic, 25 (3.7%). Carcinoma was diagnosed by CB in 99.3% of the positive group, 89.0% of the suspicious group, 44.3% of the atypia group, 13.1% of the benign group, 20.0% of the papillary lesion group and 68.0% of the nondiagnostic group. A total of 44.3% of carcinoma in the atypical cytology group and all carcinomas in the benign cytology group were lobular carcinoma, low-grade ductal carcinoma or tubular carcinoma. Excluding the nondiagnostic cytology group and papillary lesions, FNA had a sensitivity of 93.9% and specificity of 95.8%. The positive predictive value was 99.2% and the negative predictive value was 73.4%. The CB diagnosis of the 680 specimens were carcinoma, 552 (81.2%); fibroadenoma, 50 (7.3%); benign, 48 (7.1%); papillary lesion with or without atypia, 16 (2.3%); atypical ductal hyperplasia, 10 (1.5%); suspicious, 3 (0.4%) and insufficient, 1 (0.2%). Three cases diagnosed by CB as suspicious but positive by FNA, and 4 cases diagnosed by CB as benign but suspicious or positive by FNA, showed invasive carcinoma in excisional biopsy in the follow-up.

CONCLUSION: FNA is a sensitive and specific diagnostic tool in comparison to CB. FNA can be used to complement CB in the diagnosis of radiologic nonbenign breast lesions.

From the Department of Pathology, British Columbia Cancer Agency, and Department of Radiology, University of British Columbia, Vancouver, British Columbia, Canada.

A-35 CYTOLOGIC FEATURES OF BENIGN VS. MALIGNANT PAPILLARY NEOPLASMS OF THE BREAST

A. Pogacnik

OBJECTIVE: To determine the cytomorphologic features of benign papillomas and papillary carcinomas of the breast from fine needle aspiration biopsy (FNAB) samples and find the differences between the neoplastic processes.

METHODS: The files in the Department of Pathology and Cytopathology at the Institute of Oncology, Ljubljana, Slovenia, were searched through the computer database for breast FNABs with subsequent histology confirming papilloma or papillary carcinoma. From January 2000 to March 2003, 41 histologically verified papillomas were found (original cytomorphologic diagnoses were 8 papillomas, 15 papillary neoplasms, 7 proliferative fibrocystic diseases, 10 benign lesions not otherwise specified) and 38 papillary carcinomas (original cytomorphologic diagnoses were 14 papillary neoplasms, cannot exclude papillary carcinoma; 22 carcinomas; 2 unsatisfactory samples because of the presence of old blood). All FNAB slides were reviewed for cellularity, papillary groups, single epithelial cells, microcalcifications, nuclear atypia and presence of the hemosiderin-laden macrophages.

RESULTS: Markedly increased cellularity was present in 7 of 41 papillomas and 21 of 38 papillary carcinomas, papillary groups in 19 of 41 papillomas and 24 of 38 carcinomas, dissociated single epithelial cells in 11 of 41 papillomas and 23 of 38 carcinomas, microcalcifications in 8 of 41 papillomas and 8 of 38 carcinomas, atypia in 2 of 41 papillomas and 21 of 38 carcinomas and hemosiderin-laden macrophages in 25 of 41 papillomas and 18 of 38 carcinomas.

CONCLUSION: We conclude that markedly increased cellularity and dissociated single cells together with nuclear atypia favor a diagnosis of papillary carcinoma. On the other hand, the distinction between papilloma and low nuclear grade papillary carcinoma cannot be made reliably on cytologic features.
GENOMICS IN CYTOLOGY

A-37 APPLICATION OF TWO-DIMENSIONAL DIFFERENTIAL ASSURANCE IN GEL ELECTROPHORESIS PROTEOMIC TECHNIQUE TO PROFILE VARIOUS SUBTYPES OF HISTOPATHOLOGIC BREAST CANCERS

R. Murray, S. Stansfield, S. Kruger, J. Harris, T. Walsh, A. Jones, I. Bennett, D. Papadimos, A. Herington and L. Chopin

OBJECTIVE: Morphologic evaluation of breast tumors provides limited prognostic information. Markers that may assist in the prediction of the risk of metastasis would be helpful. Changes in mRNA expression do not necessarily correlate with protein expression, and therefore the functional relevance of gene microarrays is questionable. Proteomic expression profiling has potentially greater clinical application, allowing the identification of target proteins and the production of corresponding antibodies. Few studies have applied two-dimensional differential in gel electrophoresis (2-D DIGE) proteomics to well-characterized breast cancer samples.

MATERIALS AND METHODS: Clinical samples were chosen based on the histologic classification of the tumor. The 2-D DIGE method was used to investigate differential protein expression among cancer samples. This new system is superior to standard 2D-PAGE and represents a major new advance in accuracy and reproducibility. Imaging and analysis was performed using a Typhoon Imager, and DeCyder and Same Spots (Nonlinear) software. Differentially expressed proteins were identified using tandem mass spectrometry and the Mascot program.

RESULTS: Proteins that are differentially expressed between normal and cancerous cultured breast epithelial cell lines have been identified and the proteomic profiles of subtypes of histopathologic breast primary and secondary tumors compared. Significantly differentially expressed proteins will be validated to verify their usefulness as diagnostic markers for breast cancer.

CONCLUSIONS: We are currently identifying proteins that could be useful in characterizing specific subgroups of tumors, as well as proteins that are likely to be involved in metastasis. This translational proteomic approach allows us to identify clinically relevant target biomarkers for breast cancer, which can be applied to histologic and cytologic applications. This may lead to the identification of diagnostic markers or targets for therapeutic for breast cancer.

From the Department of Cytology, Sullivan Nicolaides Pathology Laboratory; University of Queensland; and School of Life Sciences, Queensland University of Technology, Brisbane, Queensland, Australia.

A-39 ABERRANT METHYLATION OF RASSF1A IN SMALL-SIZED LUNG ADENOCARCINOMA AND ITS RELATIONSHIP TO CLINICOPATHOLOGIC FEATURES


BACKGROUND: Aberrant methylation of CpG islands in promoter regions of tumor cells is one of the major mechanisms for silencing of tumor suppressor genes. The RAS association domain family 1A (RASSF1A) gene was isolated from the 3p21.3 region homozgyously deleted in lung cancer cell lines, and it was shown to be inactivated by hypermethylation of the promoter region in lung cancers. In this study, we investigated the clinicopathologic significance of RASSF1A methylation in the development or progression of small-sized (< 2.0 cm) lung adenocarcinoma. It is important to identify a marker for high-risk, early-stage patients who should benefit from new investigational adjuvant therapies.

METHODS: Surgically resected specimens from 77 cases of small-sized primary lung adenocarcinoma were studied. We determined the frequency of aberrant promoter methylation of the RASSF1A genes in small-sized adenocarcinoma. Aberrant promoter methylation was examined using methylation-specific polymerase chain reaction (PCR) (MSP).

RESULTS: Twenty-five of 77 (32.5%) tumors showed RASSF1A methylation. RASSF1A methylation was dominantly detected in smokers (p = 0.03), without gender, age, T stage, N stage and pathologic stage. RASSF1A methylation correlated with adverse survival by univariate analysis (p = 0.005) as well as multivariate analysis (p =
0.0062; RR 4.251; 95% CI, 1.507–11.993). Furthermore, RASSF1A promoter hypermethylation in resected stage I small-sized lung adenocarcinoma was associated with impaired patient survival (p < 0.01).

CONCLUSION: Aberrant promoter methylation of the RASSF1A was present in 25 of 77 (32.5%) of small-sized lung adenocarcinoma by MSP assay. These results indicated that epigenetic inactivation of RASSF1A plays an important role in the progression of small-sized lung adenocarcinoma, and that RASSF1A hypermethylation appears to be a useful molecular marker for the prognosis of patients with small-sized and stage I lung adenocarcinoma. RASSF1A is a potential tumor suppressor gene that undergoes epigenetic inactivation in lung adenocarcinoma through hypermethylation of its promoter region. RASSF1A methylation was significantly related to unfavorable prognosis in small-sized lung adenocarcinoma.

From Tokyo Medical University, Tokyo, Japan.

A-40 TELOMERASE ACTIVITY IN CONVENTIONAL TELOMERE REPEAT AMPLIFICATION PROTOCOL (TRAP) AND TRAP IN SITU IN EFFUSIONS FROM THE SEROUS CAVITIES

M. Hansson, N. Zendehrokh, J. Ohyashiki, K. Ohyashiki and A. Dejmek

OBJECTIVE: We have previously found TRAP in situ valuable in the diagnosis of malignancy in effusions, whereas varying sensitivities and specificities for malignancy have been reported with conventional TRAP. Therefore our aim was to elucidate the discrepancies between the methods.

MATERIALS AND METHOD: Twenty-three benign and malignant effusions were analyzed. Telomerase activity of whole cell lysate was measured with a Telo TAGGG telomerase polymerase chain reaction (PCR) enzyme-linked immunosorbent assay (ELISA) PLUS kit with modifications to exclude PCR inhibitors. TRAP in situ was performed on cytospins. The type and amount of positive cells, and their fluorescent intensities were evaluated. The proportions of different cell types were assessed in Giemsa-stained cytospins. An estimate of total TRAP in situ activity on the slide was compared with the level of telomerase activity in conventional TRAP.

RESULTS: TRAP in situ: Of malignant cases, 13 of 14 showed moderate or strong activity, 1 heavily blood-stained fluid was negative. Of benign effusions 5 of 7 were negative, 2 of 7 showed weak reactivity in monocellular cells and 2 of equivocal cases showed moderate or strong activity. TRAP: In 4 cases, no internal standard was detected, indicating the presence of PCR inhibitors. The relative telomerase activities (% of internal standard) were 33.1–72.7.

CONCLUSION: The TRAP in situ results correlate well to final diagnoses, whereas conventional TRAP does not differentiate between malignant and benign cases. The proportion of positive cells and their fluorescence intensity explain some of the discrepancies. Lymphocytes may contribute to falsely high values. Conventional TRAP also suffers severely from the presence of inhibiting substances, making it unsuitable for effusions.

A-41 SENSE AND ANTISENSE NONCODING MITOCHONDRIAL RNA DIFFERENTIATE NORMAL FROM TUMOR CELLS

E. Landerer, J. Villegas, V. Burzio, L. Villa, L. Boccado, M. Socias, F. Gaete, V. Toro, L. Burzio and C. Villota

BACKGROUND: Normal human proliferating cells express a sense noncoding mitochondrial RNA (sense ncmtRNA) containing an inverted repeat (IR) of 815 nts linked to the 5′ of the 16S mtRNA. These cells also express antisense transcripts with IR linked to the 5′ of the antisense 16S mtRNA (antisense ncmtRNA). In striking contrast, tumor cells overexpress the sense ncmtRNA and down-regulate the antisense transcripts. In situ hybridization (ISH) revealed that the antisense transcripts are practically absent in tumor cells present in biopsies of breast, prostate, lung, colon, stomach, cervix, brain, ovary, melanoma and lymphoma. Therefore, it seems that down-regulation of the antisense transcripts might be a universal property of cancer cells. The detection of these transcripts offers a new diagnostic method to identify cancer cells. Immortalization of human foreskin keratinocytes (HFK) with human papillomavirus (HPV) 16 or 18, also induces inhibition of the antisense ncmtRNAs expression. However, these cells also express a new sense ncmtRNA which is not present in HFK or in tumor cells. The expression of these transcripts was determined by ISH in HPV-immortalized HFK cells and in Pap smears of patients with different cytology. The sense chRNA-1 is expressed in different stages of HPV-induced transformation in raft cultures. In the same samples, the antisense ncmtRNAs were down-regulated. The sense ncmtRNA-2 is expressed in early stages of HPV-induced transformation. Pap smears from patients with abnormal cytology (CIN I–III) expressed the sense ncmtRNA-1 but not the antisense transcripts. The sense ncmtRNA-2 is expressed in CIN I and II but not in CIN III.

CONCLUSION: These results suggest that detection of the sense ncmtRNA-2 could be a powerful diagnostic method for the early detection of HPV-induced transformation in cervical cytology.

From BiosChile, Clinica Alemana, and GINOBS, Santiago, Chile.

Supported by ICM P04-071-F, FONDEF D04I1338, and a grant from Universidad Andres Bello.

A-42 MOLECULAR BIOLOGY AND KARYOTYPING ANALYSES USING FINE NEEDLE ASPIRATION MATERIAL AS A DIAGNOSTIC METHOD IN PPNET-EWING SARCOMA: INSTITUT CURIE EXPERIENCE


Between 1992 and 2005, 42 patients with Ewing sarcoma (ES) were
initially diagnosed cytologically, then biopsied at our Institute. Patient age ranged from 0 to 38 years (median 15). Fine needle aspiration (FNA) specimens were smeared for cytodiagnosis and collected in culture medium supplemented with EDTA, counted and sent for ancillary techniques, that is, molecular biology and karyotyping analyses. Karyotyping was performed only in cases in which the sample was richer than 6 million cells. Molecular analyses by reverse transcriptase polymerase chain reaction (RT-PCR) were performed in systematic research of specific translocations of ES (EWS/FLI1, EWS/ERG, EWS/11AF, EWS/FEV and EWS/ETV1), alveolar rhabdomyosarcoma (PAX3/FKHR and PAX7/FKHR), synovial sarcoma (SYT/SSX1, SYT/SSX2, SYT/SSX4) and desmoplastic small round cell tumor (EWS/WT1). Cytologic diagnoses, before ancillary techniques, results were: ES, 38 (90.7%); neuroblastoma, 2 (4.7%); and rhabdomyosarcoma (2.3%) and round cell sarcoma (2.3%), 1 case each. Molecular biology was done in 39 tumors; 34 were ES+ (specific fusion transcript detected) and 5 were ES− (no specific transcript detected). Karyotyping was performed in 16 tumors; 13 showed the characteristic t(11;22) translocation and 1 a variant t(2;22), and 3 tumors were negative. In the group of 38 tumors diagnosed cytologically as ES, ancillary techniques were ES+ in 32 tumors, ES− in 3 tumors. In the group of 4 tumors diagnosed as other malignancy, ancillary techniques showed ES+ in 2 and ES− in other 2 tumors. Two cases, however, diagnosed cytologically as other malignancies (neuroblastoma and rhabdomyosarcoma, 1 case each) were negative using ancillary techniques, but were confirmed as ES on histology specimens. Our results show that cytodiagnosis combined with molecular and karyotyping techniques using cytologic material are highly accurate in the diagnosis of ES.

From Institut Curie, Paris, France.

A-43 QUANTITATIVE ANALYSIS OF MOLECULAR MARKERS MCM2, TOPO2A AND KI-67 (MIB1) IN SQUAMOUS INTRAEPITHELIAL NEOPLASIA IN LIQUID-BASED CYTOLOGY

M. D. Beccati, C. Buriani, S. Rossi, M. Pedriali and I. Nenci

OBJECTIVE: To identify biomolecular marker profiles informative for the diagnosis of HSIL+, translate these profiles into a biomolecular Pap test and set a marker threshold for the diagnosis and for the assessment of the progression risk.

MATERIALS AND METHODS: After the cytologic diagnosis and the appropriate antigen preservation measures, a reflex immunocytochemistry was performed on 76 SurePath unstained cells samples (20 NILM from daily routine, 56 atypical squamous cells [ASC–] selected with matching histology [atypical squamous cells of undetermined significance, ASCUS; 18], high-grade atypical squamous cells, ASC-H; 11; low-grade squamous intraepithelial lesion, LSIL; 10; high-grade squamous intraepithelial lesion, HSIL; 17) by means of ProEx C and MIB1 on Ventana Benchmark XT. For the quantitative analysis, the positive and negative thresholds were established with the Olympus AnalySIS technology. Then the slide was screened manually; at least 20 fields at 10× magnification were captured and evaluated on the vertical and horizontal diameter of the slides. AnalySIS captures the area (μ²) of positive and negative nuclei. The result was expressed as percentage of positive nuclei with respect to the total number of nuclei. The total number of fields captured and evaluated is > 3,000.

RESULTS: The results of quantitative analysis are correlated with the cytologic diagnosis. Median values of positivity for ProEx C are: NILM 0.00, ASCUS 0.70, LSIL 0.00, ASC-H 5.82 and HSIL 14.37. Median values of positivity for MIB1 are NILM 0.00, ASCUS 0.89, LSIL 1.80, ASC-H 9.62 and HSIL 15.25. These results are correlated.

CONCLUSION: ProEx C and MIB1 are valid proliferation markers. The quantitative threshold of 3% (ProEx C) and 5% (MIB1) of positive cells seems to be an adequate split between NILM and ASC-H+. ProEx C indicates an aberrant S-phase induction due to E6/E7 hrHPV. Consequently, while the MIB1 is a general proliferation marker, ProEx C could be a valid adjunct to a morphologic Pap test, refining it to a biomolecular Pap test for progression risk identification.

From the Department of Pathology Oncology, S. Anna University Hospital; and Department of Surgical Pathology, University of Ferrara, Ferrara, Italy.

A-44 ProEx C IMMUNOSTAIN IN THE EVALUATION OF SQUAMOUS AND GLANDULAR LESIONS IN CERVICAL SPECIMENS

S. Santati, P. Huetman and L. Ylagan

OBJECTIVE: Minichromosome maintenance (MCM2) and topoisomerase II alpha (TOP2A) proteins play an important role in the regulation of eukaryotic DNA replication and are overexpressed in a number of dysplastic and malignant tissues. ProEx C antibody is an immunocytochemical stain for TOP2A and MCM2 proteins. The purpose of our study was to evaluate ProEx C staining in dysplastic squamous and glandular lesions of the endocervix.

MATERIAL AND METHODS: A total of 145 consecutive cervical biopsies; 19 low-grade squamous intraepithelial lesion (LSIL), 25 high-grade squamous intraepithelial lesion (HSIL), 23 squamous metaplasia (SM), 15 endocervical adenocarcinoma in situ (AIS), 39 benign endocervix (BE), and 4 squamous cell carcinomas (positive controls) from loop electrosurgical excision procedure (LEEP) and hysterectomy specimens were obtained from our files. ProEx C immunohistochemical stain was performed on all cases. Squamous lesions were graded on a scale of 0–4: 0 = none, 1+ = basal, 2+ = lower 1/3, 3+ = lower 2/3, 4+ = full thickness. Endocervical lesions were graded on a scale of 0–3: 0 = none, 1+ = 1 in 20–50 cells, 2+ = 1 in 3–20 cells, 3+ diffuse strong nuclear staining. The Kruskal-Wallis test was used for statistical analysis.

Results: Twenty-two of 25 patients with HSIL, and 17 of 19 patients with LSIL had 2+ staining. All 23 patients with SM had 0–1+ staining: SM vs. LSIL, p < 0.0001; SM vs. HSIL p < 0.0001; LSIL vs. HSIL, p = 0.0055. All 14 patients with AIS had ≥ 2+ staining. Fifty-five of 59 patients with BE had 0–1+ staining, and none had 3+ staining: p < 0.0001. Sensitivity: LSIL = 85%, HSIL = 88% and AIS = 100%. Specificity: LSIL = 100%, HSIL = 100% and AIS = 78%.

CONCLUSION: ProEx C is a sensitive and specific marker for distinguishing dysplastic squamous and endocervical lesions of the cervix from benign processes. These findings in cervical biopsy specimens should prompt evaluation of ProEx C in gynecologic cy-
A-45  PREDICTING HIGH-RISK HUMAN PAPILLOMAVIRUS INFECTION, PROGRESSION OF CERVICAL INTRAEPITHELIAL NEOPLASIA AND PROGNOSIS OF CERVICAL CANCER WITH A PANEL OF 13 BIOMARKERS TESTED IN MULTIVARIATE MODELING

M. Branca, M. Ciotti, C. Giorgi, D. Santini, L. Di Bonito, S. Costa, P. Di Bonito, S. Syrjänen, C. Favalli and K. Syrjänen

OBJECTIVE: We used comprehensive multivariate models to disclose, whether any of our previously analyzed 13 markers or any panel of markers would be independent predictors of intermediate end-point markers in cervical carcinogenesis.

MATERIAL AND METHODS: A series of 150 CC and 152 cervical intraepithelial neoplasia (CIN) lesions have been examined using immunohistochemical (IHC) staining for the following biomarkers: E-cadherin, ERK-1, LR67, MMP-2, TIMP-2, NFkB, nm23-H1, p16INK4a, PCNA, Survivin, hTERT, Topo-2a and VEGF-C. Multivariate models were constructed to test predictive power of the markers for 3 outcomes: high-grade CIN, HR-HPV, CC survival. Performance indicators were calculated using the algorithm of Seed et al.31 and compared by the areas under ROC curve.

RESULTS: In multivariate modeling, 3 marker panels (Panels 1, 2 and 3) could be established, consisting of 5 independent predictors of CIN2 (E-cadherin, ERK-1, LR67, Topo-2a, VEGF-C), 3 predictors of HR-HPV (Survivin, p16INK4a and hTERT) and 2 predictors of CC survival (nm23-H1 and TIMP-2), respectively. In predicting CIN2, the best balance between SE and SP was obtained by combining the 2 most powerful predictors in Panel 1 (VEGF-C and LR67), giving the area under ROC curve 0.847 (95% CI 0.847–0.947), OR = 86.27 (95% CI 19.71–377.47), SE 86.0%, SP 93.3%, positive predictive value (PPV) 99.1% and negative predictive value (NPV) 43.1%. When translated to a screening setting (10,000 women, CIN2 prevalence 1%), this marker combination would detect CIN2 with 86.0% SE, 100% SP, 99.1% PPV and 43.1% NPV, area under ROC curve 0.930 (95% CI 0.909–0.951) and with OR = 29998.0 (95% CI 7879.0–37338.0).

CONCLUSION: Consonant with the recent consensus statement on markers in CC screening, the use of multiple markers that complement each other is likely to be the ultimate option. Combining 2 markers (LR67 and VEGF-C) enables highly accurate detection of high-grade CIN in a clinical setting. Testing the performance of this marker combination in a screening setting, however, necessitates their analysis in cytologic samples.

From Washington University, St. Louis, Missouri, U.S.A.

DIAGNOSTIC CYTOLOGY: CERVICAL CYTOLOGY, ENDOMETRIUM AND IMMUNOLOGY

A-46  MULTIVARIATE STATISTICAL STUDY FOR EFFECTIVE CRITERION DETECTING WELL-DIFFERENTIATED ADENOCARCINOMA IN ENDOMETRIAL CYTOLOGY


OBJECTIVE: The prognosis of endometrial carcinoma is relatively favorable if the tumor cells are confined exclusively within the endometrial mucosa. Early detection of endometrial carcinoma depends on the validity of diagnostic cytology. In practice, however, the validity of endometrial cytology is relatively poor, especially when attempting to discriminate well-differentiated adenocarcinoma (World Health Organization grade 1 adenocarcinoma) from hyperplastic lesions. The present study aimed to define effective criteria for discriminating G1 adenocarcinoma from benign and hyperplastic lesions in endometrial cytologic diagnosis using multivariate analysis.

MATERIALS AND METHODS: The current study consisted of 48 cases of endometrial cytology that were 11 cases of G1 adenocarcinoma, 22 cases of hyperplasia and 15 “normal” controls. Cellular clumps composed of 100 endometrial epithelial cells were selected microscopically in each case. The epithelial clumps were characterized quantitatively by a combination of 5 parameters: (1) normal clumps (tube or sheet pattern), (2) hyperplastic clumps (dilated or branched pattern), (3) abnormal clumps (spicular shaped pattern), (4) large tissue fragment 1 (high-density of epithelial clumps) and (5) large tissue fragment 2 (low-density of epithelial clumps). The data were submitted to 5-variate cluster analysis.

RESULTS: The result of 5-variate cluster analysis showed that 48 cases were classified into 3 groups that were group A, mainly composed abnormal clumps; group B, characterized by hyperplastic clumps and large tissue fragment 2; and group C, mainly composed of normal clumps.

CONCLUSION: Comparison of this classification to the histopathologic diagnosis revealed that group A corresponded to 11 cases of G1 adenocarcinoma (100%), while groups B and C corresponded to noncarcinoma. This means that G1 adenocarcinoma can be detected using the criterion obtained by the analysis. Thus the morphometric-multivariate statistical method can be of great help in improving the cytodiagnostic validity of endometrial cytology.

From the Departments of Pathology and Medicine, Fukuoka University, Chickasha Hospital, Fukuoka; and Kurashiki Central Hospital, Okayama, Japan.

A-47  CELLULAR FEATURES OF ENDOMETRIAL HYPERPLASIA AND WELL-DIFFERENTIATED ADENOCARCINOMA USING DIAGNOSTIC CRITERIA BASED ON THE CYTOARCHITECTURE OF TISSUE FRAGMENTS


BACKGROUND: Because cellular atypia is often limited in endometrial hyperplasia and well-differentiated endometrial adenocarcinoma, diagnostic criteria for endometrial cytology have not been fully established. New diagnostic criteria based on the compo-
sion and architecture of tissue fragments (cytoarchitecture) in the smears were used in the present study. Cytologic features are of less importance because the distinction between endometrial hyperplasia and grade 1 adenocarcinoma relies more on architectural features than cellular changes. The purpose of the current study was to determine the form of the cytoarchitecture that reflects the histologic structure and to examine the cellular features in endometrial hyperplasia and grade 1 adenocarcinoma.

METHODS: The frequency of each type of cell clump (tube or sheet-shaped pattern, dilated or branched pattern, irregular protrusion and papillotubular pattern) were obtained from 49 cases of normal proliferative endometrium (NPE) (patient age range, 28–51 years; average age, 39.9 years), 63 cases of endometrial hyperplasia without atypia (EH) (patient age range, 35–65 years; average age, 47.7 years), 13 cases of endometrial hyperplasia with atypia (AEH) (patient age range 47–65 years; average age, 53.8 years), and 49 cases of grade 1 adenocarcinoma (patient age range, 42–73 years; average age, 58.9 years).

RESULTS: Certain characteristics of the cytoarchitecture were observed. In the NPE, cell clumps with a tube or sheet-shaped pattern were found in 97.5% of cases. In the EH, cell clumps with a dilated or branched pattern were found in 34.9% of cases. In the grade 1 adenocarcinoma, cell clumps with irregular protrusions were found in 61.8% cases, whereas a papillotubular pattern was present in 29.7% of cases.

CONCLUSION: The results of the current study revealed that cytoarchitectural criteria appear to be more useful for the cytologic assessment of endometrial lesions, especially for the diagnosis of endometrial hyperplasia and grade 1 adenocarcinoma.

From the Department of Pathology, Saiseikai Noe Hospital, Osaka; Department of Pathology, Kurashiki Central Hospital, Okayama; Department of Pathology, Saiseikai Shiga Hospital, Shiga; Department of Pathology, Tohoku University, Sendai; and Department of Chemistry and Life Science, A. Kanbour-Shakir, British Columbia Cancer Agency, Vancouver, British Columbia, Canada.

A-48 CYTOHISTOLOGIC CORRELATION OF ENDOMETRIAL CELLS IN PAP SMEARS OF WOMEN 40 YEARS AND OLDER

A. Kanbour, E. Elishaev, A. Gabala, J. Modery and A. Kanbour-Shakir

BACKGROUND: The Bethesda 2001 Reporting System for cervical cytology states that the presence of cytologically benign-appearing endometrial cells in women 40 years or older should be reported under the general category of “other.” This would allow clinicians to investigate further and perform an endometrial sampling, if clinically indicated. We aim to determine the clinical significance of benign-appearing endometrial cells in Pap smears in women 40 years or older and correlate this finding with menstrual cycle, menopausal status and subsequent endometrial histology.

METHODS: A database search of Pap tests with benign endometrial cells in patients 40 years and above was performed at the Magee-Womens Hospital of the UPMC Health System Cytology Department, from January 1 to July 1, 2005. A total of 568 cases were identified, of which 252 (44.5%) had subsequent endometrial sampling within 6 months.

RESULTS: Only 6 of 252 (2%) showed significant histopathologic abnormalities. All were from 80 of 252 (7.5%) postmenopausal women, of which 3 presented with abnormal bleeding. The endometrial sampling findings were as follows: endometrial adenocarcinoma in 4 (3 invasive and 1 endometrial intraepithelial carcinoma [EIC]) and atypical complex hyperplasia in 2. The remaining Pap tests (172 of 252 [68%]) were collected from premenopausal women. Of those, 103 (60%) were obtained within the first 12 days of the cycle. Their endometrial sampling findings were as follows: complex hyperplasia (without atypia) in 4, endometrial polyp in 47, disordered proliferative endometrium in 46 and benign endometrium in 75.

CONCLUSION: Our study showed that 7.5% of Pap tests from postmenopausal women had significant endometrial pathology. Therefore, in premenopausal women, clinical correlation is recommended. The appropriate clinical follow-up is up to the treating clinician who has all of the patients’ pertinent clinical information. Whereas, in premenopausal women, endometrial cells can normally be seen in Pap test during the first 12 days of cycle (menstrual or early proliferative phase) and are usually not associated with endometrial pathology. It is important to report the calculated date of menstrual cycle when possible, to reduce unnecessary endometrial sampling in this group.

From Magee-Womens Hospital of University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, U.S.A..

A-49 THE RISK OF SQUAMOUS INTRAEPITHELIAL LESION IN WOMEN WHO HAVE PERSISTENT HYPERKERATOSIS OR TYPICAL PARAKERATOSIS IN CERVICAL CYTOLOGY

C. Zhou and M. Hayes

OBJECTIVE: Hyperkeratosis and typical parakeratosis in an otherwise negative cervical Pap smear are classified as negative for intraepithelial lesion or malignancy in The Bethesda System for Reporting Cervical Cytology. However, persistent hyperkeratosis and typical parakeratosis may be associated with an increased risk of cervical squamous intraepithelial lesion. The current study is to evaluate such risk.

METHODS: A total of 100 women who had persistent hyperkeratosis or typical parakeratosis were negative for cervical squamous intraepithelial lesion over 3 years and who had at least 1 colposcopic biopsy during the period were identified from the Cervical Cancer Screening Program, British Columbia Cancer Agency.

RESULTS: Colsoscopic biopsy results of the 100 women who had persistent hyperkeratosis or typical parakeratosis were negative, 87; condyloplasia, 3; mild dysplasia, 8; moderate dysplasia, 2. No severe dysplasia or squamous cell carcinoma in situ was seen histologically. Women with a previous history of condyloplasia or biopsy-proven cervical high-grade squamous intraepithelial lesion (HSIL) had low-grade squamous intraepithelial lesion (LSIL) (condyloplasia or mild dysplasia) in 19.4% (7 of 36) and HSIL (moderate dysplasia) in 5.3% (2 of 36) in the biopsy. Women with a negative history had LSIL (condyloplasia or mild dysplasia) in only 6.2% (4 of 64) and none had HSIL on biopsy. The difference had statistical significance (p < 0.05). Women with a previous history of cervical squamous intraepithelial lesion and persistent hyperkeratosis or typical parakeratosis in cervical cytology had an increased risk of LSIL and HSIL in their cervical biopsy.

CONCLUSION: Among women who have persistent hyperkeratosis or parakeratosis in cervical cytology, those women with a history of previous cervical squamous intraepithelial lesion need colposcopy and those without such a history can be followed by annual Pap smear.

From the Department of Pathology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada.
A-50 CYTOLOGIC DIAGNOSIS OF LYMPHOMA IN SEROUS EFFUSIONS
G. Cunha Santos, S. MacDonald, S. Boerner and W. Geddie

OBJECTIVE: The cytodiagnosis of malignant lymphoma in serous effusions can be challenging, and classification of lymphomas by cytotologic means in extranodal sites is controversial. This study was undertaken to evaluate how often specific classification of lymphoma was achieved in serous effusions using a combination of morphology and immunophenotyping.

METHODS: Case files from 2002 to 2006 were searched for cases of serous effusion on which an unequivocal diagnosis of lymphoma was rendered. Cases were reviewed for the completeness of investigation including morphologic assessment and immunoprofiling to determine the contribution of these parameters to specific lymphoma classification.

RESULTS: There were 67 confirmed cases of lymphoma in 56 pleural and 11 peritoneal fluids from 45 patients (19 women, 26 men). Immunophenotypic analysis (laser scanning cytometry, flow cytometry, immunocytochemistry), molecular analysis, or a combination of these techniques was triggered by morphologic assessment and performed in a majority of cases. Cytologic diagnoses rendered for patients were 17 (37.8%) large cell lymphoma, not otherwise specified (NOS); 11 (24.4%) large B-cell lymphoma; 3 (6.7%) small B-cell non-Hodgkin lymphoma; 4 (8.9%) small lymphocytic lymphoma; 2 (4.4%) follicular lymphoma; 2 (4.4%) Burkitt’s lymphoma; 2 (4.4%) peripheral T-cell lymphoma; 1 (2.2%) primary effusion lymphoma; 2 (4.4%) non-Hodgkin lymphoma (NHL) NOS; and 1 (2.2%) T-Cell lymphoblastic lymphoma or leukemia (T-ALL). Lymphoma diagnosis without subtype was usually associated with incomplete immunophenotypic data due to inadequate or inappropriate material, but occasionally was due to ambiguous immunophenotype in marginal zone lymphomas or unsuccessful immunophenotyping due to antigen loss.

CONCLUSION: Using a combination of morphologic criteria and ancillary investigations specific lymphoma subtype diagnosis is possible in serous effusions. Successful ancillary studies are dependent on history or clinician request triggering rapid assessment screening of samples so that material can be optimized for appropriate studies.

From the University of Toronto, Toronto, Ontario, Canada.

A-51 THIS ABSTRACT WITHDRAWN FROM THE PROCEEDINGS

A-52 CYTOMORPHOLOGY OF EPITHELIAL CELLS ON CONVENTIONAL CERVICAL SMEARS FROM HUMAN IMMUNODEFICIENCY VIRUS-INFECTED WOMEN
G. M. Learmonth, K. M. Pillay, F. Venter and F. Gopolang

The detailed cytomorphicologic changes of cells on conventional cervical smears from human immunodeficiency virus (HIV)-positive women have not been intensively studied or documented. It has been suggested that concurrent infection by HIV, human papillomavirus (HPV), and other sexually transmitted diseases make the accurate diagnosis and management of cervical intraepithelial lesions difficult. In a retrospective pilot study, 175 smears from HIV-positive women at Groote Schuur Hospital Cape Town, were reviewed. A total of 16 atypical squamous cells of undetermined significance (ASCUS), 50 low-grade squamous intraepithelial lesions (LSILs), 22 high-grade squamous intraepithelial lesions (HSILs) and 1 squamous carcinoma were found. Three were unsatisfactory and 84 were normal or reactive. Prominent abnormal cytologic features were noted: macronuclear cells (23×133 μm), intense widespread orangeophilia, multinucleation, phagocytosis, engulfed parabasal cells and cells with “dendritic processes.” Florid sexually transmitted infections and retroviral drugs could also cause some of these changes. Spermatozoa were noted on several smears. Causes for macrocytosis may be HPV infection, vitamin B6 or B12 deficiency, pregnancy and retroviral effect. Macrocysts are seen in bone marrow and brain smears in association with HIV infection. Intense orangeophilia (keratinization) in bizarre cells is seen in vitamin A and E deficiency. HIV-infected patients suffer from multiple vitamin deficiencies that are resistant to dietary vitamin therapy. Recognition, study and discussion of these exaggerated cellular changes may be useful in reducing inappropriate referrals to colposcopy and poor histologic correlation. HIV and acquired immune deficiency syndrome (AIDS) is the only killer epidemic infection in human history that we can study while the patients are still relatively well. Cellular material from the cervix from these women yields valuable biologic material for ongoing studies in molecular biology, virology, microbiology and development of vaccines.

From the Voume Parant Cytology Laboratory, University of Cape Town, Cape Town, and Medical Research Council Promec, Bellville, South Africa.

A-53 CELL-MEDIATED IMMUNITY AND ENERGY PRODUCTION DEFECTS IN PRECANCEROUS CERVICAL LESIONS
J. Kobilkova, A. Jandova and J. Pokorny

OBJECTIVE: Comparison of cell-mediated immunity failure using different antigens with cytology and histology of cervical lesions might indicate mitochondrial energy production defects in progress of human papillomavirus (HPV) infection.

MATERIALS AND METHODS: Pap smears, histology, and colposcopy methods are used for diagnosis of precancerous cervical lesions. In vitro leukocyte adherence inhibition (LAI) assay based on observation of T lymphocyte adherence to substrates is understood to correlate with cell-mediated immunity. T lymphocytes prepared from blood taken from healthy women and precancerous patients were examined. Effects of specific (prepared from corresponding tumor) and of nonspecific (prepared from blood of mice infected with LDV virus invading energy production system in cells) antigens on cell-mediated immunity were evaluated and compared.

RESULTS: Immunity failure is displayed in the majority of precancerous cases. The average number of nonadherent T lymphocytes with specific and nonspecific antigens are 67% and 79% (low-grade squamous intraepithelial lesion [LSIL]) and 68% and 85% (high-grade squamous intraepithelial lesion [HSIL]), respectively. Effects of nonspecific (specific) antigen correspond to (are smaller than) those observed in all types of cancer investigated.1,2 We may conclude that LDV and HPV infections cause very likely similar damage to the cellular energy production system and that in precancerous stages the energy-producing system is completely transferred to the malignant type. Reduction of energy excitation of en-
RESULTS: After we returned to Kampala, the slides were saved and restained for a final cytologic diagnosis for immediate microscopy and a preliminary cytologic diagnosis. All was performed. Smears were prepared, and selected slides stained with a mobile FNAC clinic in Hoima, in rural western Uganda, during the first time, 2 accompanying pathologists assessed patients referred to the FNAC clinic. We brought all the required materials and equipment, including a double-headed microscope. Patients were examined with nonspecific antigen does not differ from that of cancer patients. Malignant transformation of glycolytic and mitochondrial energy production systems is very likely completed to start invasive growth.

References

From the First Medical Faculty, Charles University, and Academy of Sciences of Czech Republic, Prague, Czech Republic.

A-54 A MOBILE FINE NEEDLE ASPIRATION CYTOLOGY CLINIC IN RURAL UGANDA

I. Roy and E. Othieno

OBJECTIVE: To illustrate the value of fine needle aspiration cytology (FNAC) in providing pathologic diagnoses in a low-resource rural setting of a developing nation.

MATERIALS AND METHODS: We present our experience with a mobile FNAC clinic in Hoima, in rural western Uganda, during the annual volunteer surgical camp of the Association of Surgeons of Uganda. In the past, patients were treated without pathologic diagnoses, since this service was unavailable. In 2003, for the first time, 2 accompanying pathologists assessed patients referred to the FNAC clinic. We brought all the required materials and equipment, including a double-headed microscope. Patients were examined with nonspecific antigen does not differ from that of cancer patients. Malignant transformation of glycolytic and mitochondrial energy production systems is very likely completed to start invasive growth. All were saved and restained for a final cytologic diagnosis as we returned to Kampala.

RESULTS: A total of 28 patients were assessed. Aspirated sites included soft tissue (9), thyroid (6), skin (3), breast (3), male genitalia (3), abdomen (2) and lymph nodes (1). Many lesions were advanced, reflecting the inaccessibility of adequate medical care. FNAC usually supported the clinical impression. Occasionally, it yielded clinically unexpected diagnoses, such as metastatic melanoma, low-grade cutaneous sarcoma and tuberculosis epididymitis. This last diagnosis was made intraoperatively and resulted in surgical sparing of the testis. Most final cytologic diagnoses agreed with the preliminary cytologic diagnoses (96%). Follow-up surgical specimens were available in 2 patients. In one of them, an ulcerating lactating adenoma of ectopic breast was overdiagnosed cytologically as carcinoma. In the other, a large scalp tumor was diagnosed cytologically as a low-grade cutaneous sarcoma, with subsequent histologic confirmation.

CONCLUSION: Our experience in Uganda shows that a mobile FNAC clinic can play an important role in providing pathologic diagnoses in low-resource rural settings of developing nations.

From St. Mary’s Hospital, Kitchener, Ontario, Canada; and Mulago Hospital, Kampala, Uganda.

A-55 IMPACT OF MICROPAPILLARY CLUSTERS ON LUNG CANCER TREATMENT

Y. Satoh, R. Hoshi, M. Tsuzuku, T. Horai and Y. Ishikawa

BACKGROUND: Cytologic micropapillary clusters (MPCs) are round, 3-D and cohesive clusters of cancer cells with a pseudopapillary configuration. Recently, we clearly demonstrated that MPCs from early-stage lung adenocarcinomas can be considered useful aids to assessing prognosis. We here demonstrate the clinical impact of MPCs on lung cancer treatment.

STUDY DESIGN: Study I examined MPCs and prognosis of early-stage lung adenocarcinomas. We reviewed the clinical course, preoperative cytologic specimens and histologic materials in 110 cases of stage I lung adenocarcinomas presenting during 1986–1995. The frequency of MPCs on the slides was investigated, and patients were subclassified into MPC-positive and -negative groups. Survival of the latter for 5 years was 91%, whereas in the positive group it was 76%, the difference being statistically significant. Thus the MPC is a distinct prognostic marker for early-stage lung adenocarcinomas. Study II examined MPCs and the response of lung adenocarcinomas to gefitinib. Between June 2002 and October 2003, 36 adenocarcinoma patients who experienced a relapse after surgical resection were treated with gefitinib. Associations between the response to gefitinib and cytologic characteristics were then investigated. As a result, a significant inverse link between gefitinib efficacy and MPCs was observed (p = 0.0021). Study III examined the surgical impact of MPCs in preoperative cytologic specimens. We reviewed the metastatic status of dissected lymph nodes and presence of MPCs on cytologic specimens in 203 cases with “clinical” stage I lung adenocarcinomas undergoing surgical resection during 2000–2006. As a result, significant associations between the existence of MPCs and the frequent lymph node metastasis and vascular infiltration on pathologic examination were observed (p < 0.05).

CONCLUSION: Preoperative detection of MPCs, therefore, should alert the surgeon to the necessity for radical lymph node dissection and the clinician to the need for close follow-up and use of gefitinib in patients with recurrence.

From the Departments of Thoracic Surgical Oncology, Cytology and Pathology, Cancer Institute Hospital, Tokyo, Japan.

A-56 CYTOLOGIC FEATURES OF PULMONARY FEATURES OF PULMONARY LARGE CELL NEUROENDOCRINE CARCINOMA: COMPARISON WITH SMALL CELL CARCINOMA

Y. Fujita, T. Hirose, Y. Nishigaki, M. Shimura, Y. Yamamoto, A. Takeda, Y. Yamazaki, T. Fujikane and S. Fujiiuchi

OBJECTIVE: The 1999 World Health Organization classification of lung and pleural tumors described large cell neuroendocrine carcinoma (LCNEC) as a subtype of neuroendocrine tumors, although it was subcategorized as a type of large cell carcinoma. However, it is difficult to diagnose LCNEC pathologically and cytologically. The purpose of present study was to establish cytologic features of pulmonary LCNEC.
MATERIALS AND METHODS: Samples from 10 pulmonary LCNECs (7 bronchial brush, 2 imprint, and 1 fine needle aspiration [FNA]) and 10 small cell lung carcinomas (SCLCs) (3 bronchial brush, 4 imprint, 2 fine needle aspiration, 1 transbronchial aspiration) confirmed histologically were analyzed. The cytologic findings of LCNEC were compared with those of SCLC.

RESULTS: The original cytologic diagnosis of the cases with LCNEC was LCNEC in 2 of the cases while the remaining 4 were misdiagnosed as SCLC, 3 as squamous cell carcinoma, and 1 as adenocarcinoma. The original cytologic diagnosis was SCLC in all of the cases with SCLC. Cytologic findings of LCNEC compared with those of SCLC were as follows. Many clusters of tumor cells were found, which comprised intermediate to large-sized and round or polygonal cells with relatively abundant cytoplasmas. The clusters were often 3-D. Tumor cells had hyperchromatic nuclei and showed an increased nucleus to cytoplasm ratio. One or 2 prominent nucleoli were observed. Chromatin formation was finely or coarsely granular. Rosette formation was sometimes observed. Single-file pattern and crush artifact were observed less frequently.

CONCLUSION: Cytologic features that may be useful for the diagnosis of LCNEC are the presence of prominent nucleoli, intermediate to large nuclear size, abundant cytoplasms, 3-D clusters and lack of single-file pattern. It is possible to differentiate LCNEC from SCLC preoperatively by cytologic characterization if a sufficient number of tumor cells are obtained.

From Dohoku National Hospital, Hokkaido, Japan.

A-57 EPIDERMAL GROWTH FACTOR RECEPTOR GENE IN FINE NEEDLE ASPIRATES FROM METASTATIC SITES OF NON–SMALL CELL LUNG CANCER

C. Bozzetti, R. Nizzoli, A. Guazzi, M. Tiseo, F. Leonardi, D. Gasparro, V. Franciosi and A. Ardizzoni

OBJECTIVE: Recent studies have suggested that epidermal growth factor receptor (EGFR) gene copy number obtained by fluorescence in situ hybridization (FISH) should be used to predict which non–small cell lung cancer (NSCLC) patients are expected to respond to anti-EGFR treatments. However, it is still unknown whether EGFR status differs in metastases compared to primary NSCLC. Increased copy number of the HER2 gene seems also associated with sensitivity in anti-EGFR treatments in EGFR-positive patients, supporting use of HER2 FISH analysis for selection of patients for anti-EGFR treatments. In all studies FISH has been performed on histologic material. The possibility to perform FISH analysis on cytologic material obtained by fine needle aspiration from superficial and deep metastases would allow assessment of the EGFR and HER2 status whenever the metastatic site is not accessible to biopsy.

MATERIALS AND METHODS: EGFR and HER2 gene copy number was analyzed by FISH on fine needle aspirates (FNAs) obtained from 16 patients with metastatic NSCLC (11 lymph node, 2 liver, 1 abdomen, 1 skin and 1 peritoneal effusion).

RESULTS: Of the 16 cases assessed by EGFR-FISH, 12 had ≥ 4 EGFR copy number. 1 Of the 9 cases assessed by HER2-FISH, 6 had ≥ 4 HER2 copy number. All 16 cases showed a homogeneous cell population concerning EGFR and HER2 copy number, except for 1 case showing a heterogeneous cell population partly having ≥ 4 EGFR copy number and partly < 4 copy number.

CONCLUSION: EGFR and HER2 FISH can be reliably assessed on FNAs obtained from NSCLC metastases. Given the observation that EGFR expression is not stable during metastatic progression in a significant proportion of NSCLC, the possibility to evaluate EGFR and HER2 by FISH on FNAs could allow for a more accurate evaluation of gene copy number on NSCLC metastases.

Reference
Cappuzzo F et al: JNCI 2005

From the Medical Oncology Unit, Parma, Italy.

A-58 IMMUNOCYTOCHEMICAL EVALUATION OF REPAIR GENES MSH6 AND MGMT IN LUNG CARCINOMAS

G. Kanellis, E. N. Politit, I. Chatzistamou, V. Kyriakidou and H. G. Koutselini

BACKGROUND: The mismatch repair system (MMR) is a DNA repair mechanism responsible for the correct propagation of the double-stranded DNA. The recognition of the mismatches is performed mainly by the MSH2-MSH6 (GTBP) heterodimer called hMutS. It is shown that the functions of the MMR system, due to promoter methylation of the genes involved (hMSH2, hMSH6), play an important role in lung carcinogenesis. Another DNA repair mechanism is the single-step repair by the MGMT gene, which is found in lung carcinomas to be inactivated due to promoter methylation.

OBJECTIVE: In our study we assessed the immunocytochemical expression pattern of the mismatch repair protein MSH6 and the MGMT protein of the 1-step DNA repair mechanism in various histologic types of primary lung carcinomas.

MATERIALS AND METHODS: FNA specimens from 88 patients, aged 41–81 years (mean age 65), with primary lung carcinomas (26 squamous cell carcinomas, 52 adenocarcinomas and 10 small cell carcinomas) were included in our study. We performed a biotin-streptavidin-peroxidase immunocytochemical method using specific monoclonal antibodies for MSH6 and MGMT (clones H-141 and N-16, respectively).

RESULTS: Six of 26 squamous cell carcinomas (23.1%), 6 of 52 adenocarcinomas (11.5%) and 4 of 10 (40%) small cell carcinomas showed MSH6 expression. Similarly, 2 of 26 squamous cell carcinomas (7.7%), 4 of 52 adenocarcinomas (7.7%) and 6 of 10 (60%) small cell carcinomas showed MGMT expression.

CONCLUSION: The current study indicates that a deficiency of the MMR resulting in a MSH6 protein inactivation is to a great extent not related with the pathogenesis of small cell lung carcinomas; however, it seems that there is a correlation between MSH6 inactivation and non–small cell lung carcinoma development. Likewise, the expression of the MGMT protein is frequent in small cell lung carcinomas, yet the protein’s vast inactivation in both squamous cell carcinomas and adenocarcinomas suggests an important role of the inactivated MGMT gene in lung carcinogenesis.

From the Department of Cytopathology, Aretaioio University Hospital, Athens, Greece.
A-59 THE mRNA EXPRESSION OF SPECIFIC MARKERS FOR DISTINGUISHING BETWEEN REACTIVE MESOTHELIAL CELLS AND ADENOCARCINOMA IN BODY CAVITY FLUIDS

Y. Yamamoto, K. Hata, H. Ohsaki, T. T. Yamanushi and E. Hirakawa

OBJECTIVE: The morphologic evaluation of cytologic specimens from body cavity fluids presents difficulties in the differential diagnosis between benign reactive mesothelial cells and adenocarcinoma. The aim of this study was to investigate whether a panel of 4 markers (hTERT, carcinoembryonic antigen [CEA], podoplanin and calretinin) can be used as an adjunctive tool in the differential diagnosis of reactive mesothelial cells and adenocarcinoma cells in serous effusions.

MATERIALS AND METHODS: We evaluated 47 cytologic specimens of serous effusions (23 pleural, 22 peritoneal, 1 peritoneal washing and 1 pericardial). These specimens were collected from metastatic adenocarcinoma (n = 12), inflammatory cells and reactive mesothelial cell (n = 35), respectively. The primary sites of metastatic adenocarcinoma were lung cancer (n = 3), gastric cancer (n = 3), pancreatic cancer (n = 3), liver cancer (n = 3), ovarian cancer (n = 2). The expressions of each marker mRNA were assessed by reverse transcriptase polymerase chain reaction (RT-PCR).

RESULTS: Statistical significance was found with hTERT and CEA when comparing adenocarcinoma and reactive mesothelial cells (p < 0.001). The sensitivity of hTERT and CEA for adenocarcinoma was 67% and 75%, respectively, and the specificity was 100% for both. The sensitivity of podoplanin and calretinin for reactive mesothelial cells was 54% and 26%, respectively, and the specificity was 83% for both.

CONCLUSION: CEA was the most sensitive marker for adenocarcinoma, but both of the markers for reactive mesothelial cells showed low sensitivity. Although further examination is necessary, the selection and simultaneous use of several specific markers of mesothelial and cancer cells promises to be a useful adjunct to ensure the accuracy of cytologic diagnosis.

From Kyoto University, Kyoto; and Kagawa Prefectural College of Health Sciences, Takamatsu, Japan.

A-60 RANDOMIZED COMPARISON OF TWO STAINING STRATEGIES FOR RAPID ON-SITE ANALYSIS OF TRANSBRONCHIAL NEEDLE ASPIRATES

M. Louw, A. Dacon, C. Koegelenberg, K. Brundyn, C. Wright and C. T. Bolliger

OBJECTIVE: Rapid onsite analysis (ROSE) improves yield and practical use of transbronchial needle aspiration (TBNA). This study compared 2 onsite staining strategies: (1) “budget,” a simple, 30-second, cytoplasmic stain (Diff-Quik) carried out and read by 1 person; and (2) “luxury,” a sophisticated, 3-minute stain with better resolution of nuclear features (Rapid-Papanicolaou), operated and read by 2 persons.

MATERIALS AND METHODS: We randomized 126 patients (55 ± 15 years; 60% men) to TBNA with budget ROSE (B-ROSE, n = 64) or luxury ROSE (L-ROSE, n = 64). ROSE-TBNA results were compared to the final laboratory TBNA report.

RESULTS: TBNA was positive in 99 (79%) of all patients. With 626 needle passes at 170 target sites we diagnosed non–small cell lung cancer (NSCLC) (72%), small cell lung cancer (SCLC) (9%), granulomatous disease (17%) and lymphoma (2%). In these patients, L-ROSE correctly identified positive TBNA passes in 85% (129 of 152) vs. 73% (99 of 136) with B-ROSE (p = 0.44). False negative readings were more frequent with B-ROSE than with L-ROSE (27% vs. 15%, p = 0.02). Sensitivity and specificity were 85% and 96% for L-ROSE and 73% and 92% for B-ROSE. Calculated per target site, yields were 72% for L-ROSE and 60% for B-ROSE, respectively. No false positive sites occurred with either method.

CONCLUSION: The Rapid-Papanicolaou stain is superior to Diff-Quik for yield and accuracy in ROSE-TBNA.

From Cytopathology, the Department of Pathology, NHLS, Tygerberg, South Africa; and Department of Internal Medicine, University of Stellenbosch, Bellville Campus, South Africa.

A-61 SMALL CELL LUNG CANCER: CYTOLOGIC VS. HISTOLOGIC DIAGNOSIS

J. Dontařáска-Kukawińska, K. Ruenke, B. Gornicka and M. Bogdanska

OBJECTIVE: Small cell lung cancer (SCLC) is a very aggressive neoplasm and differs significantly in the biology, course and treatment from those of non–small cell histologic types. Accurate and quick diagnosis is crucial to initiate a proper treatment. The aim of this study was to establish the value of initial cytologic diagnosis compared with histologic biopsy examination and to present typical cytologic features of SCLC.

METHODS: We reviewed 116 cases of SCLC confirmed by cytologic (bronchial brushings, pleural fluids, fine needle aspiration biopsies [FNABs]) of peripheral lung tumors, transbronchial FNAB of mediastinal lymph nodes and FNAB of supraclavicular lymph nodes or of the metastases to soft tissue and other organs. Possibility of histologic confirmation and usefulness of immunohistochemical staining (leukocyte common antigen, cytokeratins, synaptophysin and chromogranin) in SCLC diagnosis were analyzed.

RESULTS: In 77% of SCLC cases an accurate diagnosis was established only by cytology; in 27% of cases both cytologic and histologic biopsy were possible. Immunohistochemistry was helpful in 74% of cases in which histologic biopsy was taken. Histologic examination of bronchial biopsy confirmed cytologic diagnosis in 100%. Cytology of SCLC was uncertain in 13% of cases and histology in 30%. The main cause of difficulties in histologic diagnosis was crush artifacts from biopsy forceps. The morphology of SCLC cells was not uniform and often a mixture of non–small atypical cells and bronchial epithelial cells with signs of metaplasia was observed. Two cases of combined types of cancer were confirmed by histology. The main diagnostic problem was to distinguish small cell lung cancer from lymphomas, atypical carcinoid and undifferentiated non–SCLC consisting of small cells.

CONCLUSION: Diagnosis of SCLC in cytologic smears is possible, but the clinical data are necessary and differential diagnosis with other tumors of small cells have to be taken into account.

From the Departments of Pneumonology and Pathology, Medical University of Warsaw, Warsaw, Poland.
A-62  FINE NEEDLE ASPIRATION BIOPSY IN SUPERIOR VENA CAVA SYNDROME

K. Brundyn, M. Louw, C. Wright, M. Van Den Heuvel, A. Diacon and J. Theron

BACKGROUND: Superior vena cava syndrome (SVCS) is a medical and oncologic emergency that requires a definite tissue diagnosis before initiating therapy. Because diagnostic surgery is challenging due to a high risk of complications, especially bleeding, alternative diagnostic methods such as transbronchial needle aspiration (TBNA) and ultrasound-assisted transthoracic aspiration (TTNA) of pleural abutting lesions are proposed for a diagnosis. We hypothesize that minimally invasive methods such as these could obviate surgery in most cases and can provide an accurate diagnosis for therapeutic management.

METHODS: Between November 2000 and January 2005, 85 patients with SVCS were referred to the pulmonology unit after non-invasive evaluation—proved noncontributory (cytology from sputum, pleural fluid or a lymph node aspirate). TBFNA or TTNA was performed under local anesthesia.

RESULTS: Although the majority of patients had lung carcinomas (non–small cell type, 54%; small cell type, 32%), a minority of patients had lymphomas (3.5%). One of the patients had a thymoma. With 89 procedures, a diagnosis was established in 91% of patients. TBNA had an 86% yield (42 of 49 procedures) and TTNA had an 85% yield (34 of 40 procedures). Only 8 patients (9%) needed further evaluation or biopsies. Neither TBNA nor TTNA caused notable complications.

CONCLUSION: TTNA and TBNA are effective and safe methods for obtaining adequate diagnostic tissue for clinical management of SVCS patients. The minority of patients will require more invasive diagnostic methods for therapeutic purposes.

From the Department of Cytopathology, Tygerberg Hospital, NHLS and University of Stellenbosch, Tygerberg; and Department of Internal Medicine, University of Stellenbosch, Bellville Campus, South Africa.

A-63  THIS ABSTRACT WITHDRAWN FROM THE PROCEEDINGS

A-64  STANDARDIZED USE OF IMMUNOCYTOCHEMISTRY FOR DETECTION OF MOLECULAR MARKERS IN CERVICAL CANCER SCREENING: PART II

S. Sahebali, C. Depuydt, L. Moeneclaey, A. Vereecken and J. Bogers

OBJECTIVE: Cytology is valuable in cervical screening, but yields many false negatives and low reproducibility. p16INK4a expression in cervical specimens correlates with increased dysplasia and is a locator, drawing attention to immunoreactive cells. The interpreter decides whether these cells are dysplastic, using morphologic criteria. Standardization of sample processing is important for clinical implementation of molecular markers. We combined these features into an immunomorphologic assessment to identify high-grade lesions. Results were correlated with biopsy and high-risk human papillomavirus (hrHPV) testing results.

METHODS: The study population comprised 189 negative, 101 atypical squamous cells of undetermined significance (ASCUS), 107 low-grade squamous intraepithelial lesion (LSIL) and 103 high-grade squamous intraepithelial lesion (HSIL) patients. hrHPV testing and typing were performed by polymerase chain reaction (PCR) and immunocytochemistry by CINTEC kit with standardized and automated protocols. Biopsies were included if available. Nuclear score of immunoreactive cells was based on Bethesda System criteria, including increased size, hyperchromasia and irregular shape, leading to a score from 1 to 4, with high score defined as ≥3.

RESULTS: hrHPV was present in 63.5% negative, 81.4% ASCUS, 88.8% LSIL and 97.1% of HSIL cases. The amount of p16INK4a immunoreactive cells and nuclear score were increased with increased dysplasia. Full immunomorphologic assessment eliminated most metaplastic and atrophic cases. Histology was obtained for 138 cases: 3 glandular lesions, 71 cervical intraepithelial neoplasia (CIN)1 or less and 64 CIN2+. A total of 59 HSIL cases were confirmed CIN2+. Two CIN2+ cases did not contain hrHPV. A total of 25 cases with p16INK4a immunoreactivity and high nuclear score were confirmed CIN2+ (sensitivity 39.1%, specificity 90.1%). False negative cases were almost all cases with low cellularity or fixation problems.

CONCLUSION: Immunomorphologic assessment based on p16INK4a may facilitate detection of high-grade lesions. Assessment could be automated, increasing standardization of molecular marker use in cervical cancer screening, which is of utmost importance for implementation. With proper use in conjunction with morphology and HPV testing, molecular markers can be of value in cervical cancer management and monitoring of hrHPV infections.

From the University of Antwerp and Laboratory for Clinical Pathology, Antwerp, Belgium.

A-65  COMPARATIVE STUDY OF PTEN AND VEGF IMMUNOCYTOCHEMICAL EXPRESSION IN PRENEOPLASTIC AND NEOPLASTIC CERVICAL LESIONS


BACKGROUND: PTEN is a tumor suppressor gene that is frequently mutated in many human cancers and regulates the cell cycle, apoptosis and cell adhesion. PTEN appears to regulate angiogenesis under hypoxic conditions. Rare mutations of the PTEN gene have been reported in cervical cancer, but there are no reports on the simultaneous expression of PTEN and VEGF in preneoplastic cervical lesions.

OBJECTIVE: The aim of our study was to assess immunocytochemically the pattern of PTEN and VEGF expression in preneoplastic and neoplastic cervical lesions and to investigate any association between their expression.

MATERIALS AND METHODS: Immunocytochemical staining with mouse monoclonal anti-PTEN and anti-VEGF antibodies (clones 28H6 and VG1, respectively) was performed in a total of 60 patients with low-grade squamous intraepithelial lesion (LSIL) (human papillomavirus [HPV] n = 10, HPV/cervical intraepithelial
neoplasia [CIN1 n = 10], high-grade squamous intraepithelial lesion [HSIL] [CIN2 n = 10, CIN 3 n = 10] and squamous cell carcinoma (n = 20), using a biotin-streptavidin-peroxidase immunocytochemical method. All the cytologic diagnoses were histologically confirmed.

RESULTS: Reduced PTEN expression progressively increased along the continuum from LSIL to squamous carcinoma (p < 0.01), whereas VEGF expression showed a linear increase from LSIL to squamous carcinoma (p < 0.05). A significantly negative correlation was found between PTEN and VEGF expression (p < 0.05).

Conclusion: Our data suggest that loss of PTEN expression and increased levels of VEGF may play an important role in the progression of CIN. PTEN alterations seem to be a late-stage event in cervical carcinogenesis. Furthermore, VEGF expression may play a role in the up-regulation of PTEN. The meaning of our findings as far as the prognosis is concerned needs to be clarified.

---

A-66 ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE DIAGNOSIS ON CERVICOVAGINAL SMEARS AND USAGE OF ProEx C IMMUNOCYTOCHEMISTRY: CORRELATION STUDY WITH HIGH-RISK HUMAN PAPILLOMAVIRUS STATUS

M. Siddiqui

The cytologic detection of underlying high-grade cervical disease in patients with atypical squamous cells of undetermined significance (ASCUS) diagnosis may be improved with molecular markers known to be overexpressed in cervical carcinoma. ProEx C detects 2 such molecular markers, minichromosome maintenance protein 2 and topoisomerase II, which are associated with abnormal cell cycle regulation. The utility of the ProEx C marker, when compared with high-risk human papillomavirus (hrHPV) status, in PAP tests with ASCUS and atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H) cytology, is the goal of the current study. Cervical cytology specimens were collected in SurePath preservative and those with the diagnosis of ASCUS were included in the study. A second SurePath slide was also prepared and stained using the ProEx C reagent (TriPath Imaging). Nuclear staining of squamous cells was considered a positive result. Intensity of nuclear staining (scored from 1+ to 3+) was determined for each case. hrHPV testing was performed on each case. A total of 128 cases were studied (ASCUS diagnosis: n = 126, and ASC-H diagnosis: n = 2). Age ranged from 17 to 87 years. The hrHPV positive rate was 44%, and the ProEx C positive rate, when all cases with positive staining were included, was 50%. Using hrHPV status as a gold standard, the overall sensitivity of ProEx C was 84%, specificity 77%, positive predictive value 73% and negative predictive value 86%. A trend of increasing intensity of nuclear staining in hrHPV-positive cases was also seen. ProEx C staining, when compared to hrHPV status in PAP tests with ASCUS and ASC-H cytology, was both a sensitive and specific marker. Increased intensity of staining suggests a positive hr-HPV test result. ProEx C can be utilized for primary screening as well as for confirmation of hrHPV in patients with ASCUS diagnosis.

---

A-67 VISUALIZATION OF HUMAN PAPILLOMAVIRUS L1 CAPSID PROTEIN IN ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE AND MILD DYSPLASIA CASES USING A FORMALDEHYDE-FREE FIXATIVE AND THE CYTOBLOCK METHOD

P. Pothuis, E. Ouwerkerk-Noordam, R. Hilfrich, P. Holloway and M. Boon

OBJECTIVE: Since April 2005, over 30,000 ThinPrep slides have been prepared from liquid-based cytology samples to be formalin fixed with the brushes left in the vial by the clinician. Approximately 20% of the cervical material is used to prepare the ThinPrep cytology slide. The rest can be used to embed in paraffin with the CytoBlock method. Human papillomavirus (HPV) L1 capsid protein (L1) is a major target of the immune system. It has previously been shown that low and moderate dysplastic squamous lesions without immunocytochemically detectable L1 are more likely to progress than L1 positive cases. We visualized HPV L1 capsid protein in atypical squamous cells of undetermined significance (ASCUS) and mild dysplasia cases as diagnosed in ThinPrep slides.

MATERIALS AND METHODS: Between January and April 2006, 70 cases with the diagnosis of mild dysplasia on the ThinPrep slide had enough material for embedding in paraffin. We used the CytoBlock method as developed in our laboratory, using microwave technology for histoprocessing. First, 6 sections were cut and stained with the Papanicolaou method. Next, 6 more sections were cut from the block for L1 immunostaining. The ThinPrep slides and the histology sections were independently diagnosed.

RESULTS: The ThinPrep slides were of excellent morphologic quality for the diagnosis of ASCUS or mild dysplasia (low-grade squamous intraepithelial lesion [LSIL]). Twenty-four of the cases received the diagnosis ASCUS, for 11 cases the histologic diagnosis was CIN2 or CIN3 and the remaining 35 cases received the histologic diagnosis CIN1. In all cases, there were enough mini-biopsies in the sections to allow L1 classification of the cervical tissues. Twelve of the 24 ASCUS, 16 of 35 CIN1 and 9 of 11 CIN2 were L1 negative.

CONCLUSION: Since absence of the L1 correlates with progression of lesion, we suggest closer and more frequent monitoring of L1 negative women in comparison to those that are positive.

---

A-68 VISUALIZATION OF p16INK4A IN CYTOBLOCKS USING A FORMALDEHYDE-FREE FIXATIVE


OBJECTIVE: p16 is expressed in normal tissues and overexpressed in dysplastic and neoplastic lesions of the cervix and may thus be a useful diagnostic marker for the diagnosis of high-grade cervical intraepithelial neoplasia (CIN) and some low-grade CIN.
For this study, the expression of p16 was examined immunohistochemically using a formalin-free fixative and the cytoblock technique.

**MATERIALS AND METHODS:** p16 expression was examined in a group with negative cytology (n = 38), atypical squamous cells of undetermined significance (ASCUS) (n = 25), and high-grade squamous intraepithelial lesion (HSIL) (n = 16). Nuclear p16 staining was identified independently by 2 observers who were blinded to cytology diagnosis. All p16-positive cells were counted and documented. Counting was not continued once 200 positive cells were found. A nuclear score according to Wentzensen et al1 was applied to p16-positive squamous cells.

**RESULTS:** A mean of 0, 3, and 200 p16-positive cells were found for the group with negative cytology, ASCUS, and HSIL, respectively. A mean Wentzensen score of 0, 1.5 and 3.5 was found for the same diagnostic groups. We demonstrated that p16 expression is increasingly expressed in cell clusters and mini-biopsies of increasing grade of neoplasia. We further noted the valuable use of a formalin-free fixative and the use of the cytoblock, both of which improve the microscopical images, thereby decreasing intraobserver and interobserver variability.

**CONCLUSION:** The use of a formaldehyde-free fixative and the cytoblock technique improves microscope imaging and allows for further examination of parallel sections with markers such as Ki-67 (proliferation) or human papillomavirus (HPV) L1 capsid protein without having the patient return.

Reference

A-69 IMMUNOCYTOCHEMISTRY IN DIAGNOSTIC FINE NEEDLE ASPIRATION CYTOLOGY: APPLICATION OF A LIMITED IMMUNOCYTOCHEMICAL PANEL

P. Ganjei-Azar

**BACKGROUND:** Fine needle aspiration (FNA) cytology is now an important tool in the primary diagnosis of a number of neoplastic diseases, and therefore ancillary tests such as immunocytochemistry are being increasingly performed on FNA samples. When the specimen is adequate for cell block preparation the use of a large immuno-cytological (ICC) panel is not a problem. However, frequently, the diagnostic samples are small, usually made up of a few smears, preventing the use of a complete ICC panel. We therefore studied the value of a differential diagnosis-driven ICC approach using only 1 or 2 antibodies on Papanicolaou-stained smears.

**STUDY DESIGN:** Alcohol- or formalin-fixed, Papanicolaou-stained FNA smears from 494 patients with suspected or confirmed neoplastic disease were reviewed. In 208 of the cases cytology alone could not establish the definitive classification of tumor. In those cases, based on the clinical information and cytomorphology, the differential diagnoses were narrowed down to 2 or 3 possibilities. We then utilized a limited antibody panel to characterize the tumor. ICC was directly performed on Papanicolaou smears without destaining.

**RESULTS:** An average of 1.6 antibodies was used per specimen. In 129 (62%) cases a definitive classification of tumor was established: lymphoma, melanoma, metastatic mammary carcinoma, renal cell carcinoma, adenocarcinoma, neoplasm, etc. In 56 (27%) cases the diagnosis could be narrowed: adenocarcinoma consistent with an upper gastrointestinal or pancreatobiliary primary. In the remaining 23 (11%) cases ICC was not useful at all.

**CONCLUSION:** A nonalgorithmic, differential diagnosis-driven, limited ICC antibody panel resolves most diagnostic problems in FNA smears. This is particularly important when the sample is inadequate for cell block preparation. The procedure is simple and does not require any modification of the routine ICC staining protocol.

From the University of Miami, Miami, Florida, U.S.A.

A-70 THE EFFECTS OF TIME DELAYS ON THE IMMUNOPHENOTYPE OBTAINED BY LASER SCANNING CYTOMETRY OF CYTOLOGIC SAMPLES

S. MacDonald, W. Geddie and S. Boerner

**OBJECTIVE:** Immunophenotyping is a significant adjunct to morphologic evaluation of cytologic specimens containing lymphoid populations. Occasionally, immunophenotyping is delayed, but the effects of this time delay on the immunoprofile of lymphoid cells in cytologic samples are poorly understood. This study was undertaken to determine the effects of time and storage temperature on the immunophenotype obtained by laser scanning cytometry of lymphoid cells in cytologic samples.

**MATERIALS AND METHODS:** Immunophenotyping was performed by laser scanning cytometry on aliquots of 4 lymphoid serous effusions (2 reactive and 1 monoclonal B-cell) and 3 fine needle aspiration (FNA) samples of lymph node (2 reactive and 1 monoclonal B-cell) upon receipt and at time intervals over an 8-day period. Samples were stored at room temperature (20°C) or refrigerated (4°C), with the effusions samples unaltered and FNA samples maintained in sterile saline. The delayed immunophenotype was compared to that obtained upon receipt.

**RESULTS:** A monoclonal B-cell phenotype was detectable in effusion samples stored at either 20°C or 4°C up to 7 days following sample collection, although cell loss occurred over this time interval. Refrigeration appeared to reduce cell loss, but resulted in a relative loss of T-cells compared to B-cells with an unaltered or increased CD4/CD8 ratio. FNA samples of lymph node were much more sensitive with B-cell clonality undetectable after 1 day when stored at 20°C and 5 days at 4°C post collection.

**CONCLUSION:** Lymphoid cells in effusion samples are relatively resistant to delays in immunophenotyping with B-cell clonality detectable up to 7 days post collection in samples stored at either 4°C or 20°C. Delayed immunophenotyping results in a loss of T-cells that is exacerbated by refrigeration.

From the University Health Network, Toronto, Ontario, Canada.