1495 Stromal Cell-Derived Factor-1 Expression in 351 Non-Small Cell Lung Cancers: A Tissue Microarray and Immunohistochemical Assessment of Its Relationship to Patient Survival

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Background: The alpha-chemokine stromal cell-derived factor-1 (SDF-1) plays an important role in stem cell trafficking, and several recent reports suggest a function for SDF-1 in regulating the metastasis of non-small cell lung cancer (NSCLC). Its relationship to prognosis, however, is not clear.

Design: High-density tissue microarrays containing 351 NSCLCs with more than five years of clinical follow-up were immunostained for SDF-1 (1:25, R&D Systems) using standard avidin-biotin techniques. Staining intensity was graded as 0, 1+ (weak), 2+ (moderate) or 3+ (strong), and the percentage of tumor cells staining was graded semi-quantitatively as follows: 0=no tumor cells staining, 1= 1-32% staining, 2=33-66% staining, 3=>66% staining. For each tumor, an overall percentage of cells stained was calculated as the mean of the individual percentages recorded for the three samples of each tumor in the microarray. Information about patient survival and tumor stage was obtained. Statistical analysis was performed using Pearson correlation and Kaplan-Meier analysis.

Results: Staining intensity was graded as weak, moderate, and strong in 11.9%, 51.5%, and 27.8% of the NSCLCs, respectively. 20.0% showed 1-32% of cells staining, 18.7% fell between 33 and 66%, and 52.1% of tumors showed >66% expression. Only 9.6% of the tumors were negative. For the entire cohort, strong cytoplasmic SDF-1 staining intensity correlated significantly with improved survival (p=0.03). For stage I and II adenocarcinomas, enhanced survival also correlated positively with the overall percentage of tumor cells staining (p=0.03).

Conclusions: Increased SDF-1 expression may predict better patient survival in NSCLCs. Its wide expression in NSCLCs may indicate a role for SDF-1 in the pathogenesis of these neoplasms.

1496 SV40 and Malignant Mesothelioma: A Molecular and Immunohistochemical Study of 83 Cases from USA and Two Different Regions of Turkey

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Background: Simian virus (SV40) is an oncogenic DNA virus and may act as co-carcinogens with asbestos in the etiology of malignant mesothelioma of the pleura and peritoneum. Several studies showed DNA sequences specific for SV40 in large series mostly from United States, however, a literature search did not reveal any finger prints of the virus on the environmental malignant mesothelioma cases from Turkey.

Design: In this study, we chose 40 pleural malignant mesothelioma cases from Southeastern region, 17 cases from Middle Anatolia of Turkey, as well as 26 cases from United States to detect DNA sequences by using nested polymerase chain reaction (PCR) using the primer pairs, the SV primer set directed at the LTAg gene sequence unique to SV40 and the PYV primer set directed at a sequence shared by SV40 and papovavirus strains BK and JC, respectively. The presence of DNA was established by amplification of a 250 bp product from the betaglobin gene. We also used an avidin-biotin immunoperoxidase technique to detect SV40 T antigen (TAg) in our series.

Results: All US malignant mesothelioma cases except two showed positive immunoreaction for TAg (92%), whereas 14 cases were positive from Middle Anatolia region (82%) and only 6 cases from Southern Anatolia region of Turkey (15%). The intensity of the immunostaining was stronger and more diffuse in US cases. By PCR technique, we noted SV40 DNA sequences in 13 US cases (50%), in 3 Middle Anatolia cases (18%), and in 3 Southeastern Anatolia cases (7.5%) respectively.

Conclusions: This study showed SV40 DNA sequences in environmental malignant mesothelioma cases from two different parts of Turkey, and also confirmed the high incidence of SV40-positive cases in USA. The geographical conditions, type of the inhaled asbestos fibers, and genetic characteristics may play role in the development of malignant mesothelioma in these cases as well as SV40 infection. SV40 contaminated polio vaccination may explain the source of the virus in US cases, however fails to explain the existence of the virus in Turkey and also the higher incidence in Middle Anatolia when compared with Southeastern Anatolia.

1497 Lung Adenocarcinoma with Mixed Subtypes, a Distinct Biologic Entity?

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Background: In the 1999 WHO classification of pulmonary neoplasms, adenocarcinomas with bronchioloalveolar (BAC) and invasive components are classified as "adenocarcinoma with mixed subtypes". However, the clinicopathologic features of this subtype have not been fully defined. Since the majority of small adenocarcinomas are classified in this category and represent a heterogenous group of tumors from minimally to fully invasive cancers, further refinement of the diagnostic criteria is needed. A more accurate subclassification may help define biologically distinct subgroups, thus helping to guide patient care.

Design: 164 cases of primary lung adenocarcinomas resected between 1992-2004 were reviewed and divided into 4 groups. Group I: BAC only (49); Group II: Mixed type with =5 mm invasive component (16); Group III: Mixed type with >5 mm invasive component (63); Group IV: Invasive adenocarcinoma only (36). Tumor size and lymph nodes metastasis were compared. P53 and Ki-67 staining were analyzed in 46 cases (14 from Group I; 9 from Group II; 11 from Group III; 12 from Group IV). The Ki-67 labeling index (LI) was determined by counting 500-1000 tumor cells in three high-power fields (400X) of the most highly labeled areas. P53 overexpression was defined as the presence of nuclear staining in >20% of tumor cells.

Results: Table 1 shows the clinicopathologic characteristics of each group. None of the Group I and II tumors had lymph node involvement. In contrast, 8 of 63 Group III (12.7%) lesions and 21 of 36 Group IV (75%) lesions showed lymph node involvement. The mean Ki-67 LI and frequency of p53 overexpression showed an increasing tendency from group I to IV, with values being higher in groups III and IV than in groups I and II.

Conclusions: Data shows that adenocarcinoma with mixed subtypes (groups II, III) have characteristics between BAC (group I) and invasive carcinoma (group IV), consistent with the idea that this entity is a tumor in progression from BAC to invasive cancer. A subgroup of this entity (group II) has characteristics and biologic behavior more similar to BAC, suggesting that this subgroup could be defined as a form of early invasive cancer, and may be clinically managed as such.

TABLE 1. Clinicopathologic characteristics of each histological group

Histologic subtype	p53 (% of positive lesions)	Ki-67 LI (%) (mean ± SD)	Age (mean ± SD)	Tumor size (mean ± SD) (cm)	Lymph node involvement (%)
I	8	6.2 ± 2.7	67.7 ± 8.4	1.9 ± 1.5	0
II	11	7.5 ± 3.7	65.6 ± 9.5	1.6 ± 0.8	0
III	30	21.3 ± 14.8	66.9 ± 10.2	2.3 ± 1.2	12.7
IV	44	31 ± 19.2	68.5 ± 10.5	3.1 ± 2.6	75

Quality Assurance

1498 Frozen Section (FS) Diagnosis (Dx) in Pediatric Surgical Pathology: A Decade's Experience at a Children's Hospital (CH)

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Background: FS are critical for patient care and are a key quality component in anatomic pathology. Little data exists about the use, discrepancy, and deferral rates of FS Dx in pediatric and adolescent care. The purpose of this study was to analyze indications, discrepancies, and deferrals for all FS performed at a CH during a decade.

Design: All FS Dx for 1995-2004 were reviewed for indications, discrepancies, and deferred Dx. Discrepancies were categorized into major and minor subtypes according to impact on patient care.

Results: 33,524 surgical pathology cases were accessioned, with 2,735 individual FS (8.2%). Most frequent indications included Hirschsprung disease (HD), and questions related to neoplasms (tumor detection, specimen adequacy, triage, classification, margins). 101 discrepancies (3.6%) were identified, of which 7 (0.2%) were major, with potentially significant clinical impact, and 94 (3.4%) were minor. Major discrepancies included tumor, ganglion cell, or fungal detection. Minor discrepancies involved sampling errors, reclassification of benign or malignant neoplasms without clinical consequences, tumor grading, and recognition of ganglion cells or transition zone in suspected HD. Deferrals included tumor classification from generic to specific, identification of organisms, and evaluation of lymph node biopsies for lymphoma.

Conclusions: The FS rate of 8.2% is similar in CH and general hospitals. The major discrepancy (discordance) rate is lower, which may reflect the different indications for FS at CH. HD is a major FS Dx pitfall. The deferral rate is higher in CH and may reflect how a deferred Dx is defined. Traditional definitions of deferred and discordant FS Dx should be refined to reflect increasing use of adjunct techniques, especially in tumor classification. The findings emphasize that in CH, the majority of FS are performed for HD and for tumor classification, triage, detection, and specimen adequacy, but are used infrequently to identify normal or unknown tissue, a lesion in a radiographically directed specimen, or to detect lymph node metastases. The differences in CH FS Dx underscore the importance of education in pediatric surgical pathology.

1499 Clinician Satisfaction with Complete Subspecialization in Surgical Pathology: One Year Follow-Up at the Cleveland Clinic

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Background: Prior to July 2003, the surgical pathologists of the Department of Anatomic Pathology (DAP) at the Cleveland Clinic Foundation (CCF) practiced general surgical pathology with partial subspecialization. Encouraged by subspecialized clinician groups who demanded greater efficiency and expertise, the department re-organized to a complete subspecialization model consisting of 16 services covered by groups of 2-8 pathologists. With this new model, the DAP hoped to improve clinician satisfaction with the DAP services.

Design: Cleveland Clinic physicians were surveyed before and after implementation of subspecialization in the Department of Anatomic Pathology (DAP). Physicians were asked to rate the surgical pathology reports for: 1) Accuracy, 2) Completeness, 3) Timeliness, and the surgical pathologists for 1) Capable of answering questions and 2) Meet my expectations.(Strongly agree, Agree, Disagree, Strongly Disagree). The DAP was rated for overall satisfaction (Very satisfied, Somewhat satisfied, Somewhat dissatisfied, Very dissatisfied). Surveys were mailed to 465 physicians from the Divisions of Surgery and Medicine in April 2003, two months before and one year after implementation of complete subspecialization. Completed surveys were received from 41% of physicians from Surgery and 46% of physicians from Medicine.

Results:

Percentage of Physicians Who Strongly Agree

Survey Question	Medicine		Surgery	
	2003	2004	2003	2004
Report Accuracy	46	53	49	60
Report Completeness	32	44	44	46
Report Timeliness	35	47	44	45
Pathologist Capable of Answering Questions	53	66	65	71
Pathologist Meets My Expectations	39	54	58	60

Overall Physician Satisifaction with DAP

	Medicine		Surgery	
	2003	2004	2003	2004
Very Satisfied	64	74	77	78

Conclusions: One year after re-organization to complete subspecialization of surgical pathology at CCF:

1) There is an increase in the number of physicians in both Medicine and Surgery who feel the surgical pathology reports are accurate.

2) There is an increase in the number of Medicine physicians who feel the surgical pathology reports contain all of the necessary information and are completed in a timely manner.

3) There is an increase in the number of physicians in both Medicine and Surgery who feel the surgical pathologists are capable of answering their questions and meet their expections.

4) There is a high overall satisfaction with the DAP and an increase in satisfaction with the DAP from physicians in Medicine following complete subspecialization.

1500 A Two-Tiered Statistical Analysis-Based Quality Assurance Program for HER-2 Assessment by Immunohistochemistry

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Background: Immunohistochemical (IHC) testing for HER-2 overexpression is now routinely performed on breast cancers in the US. However, the level of immunostaining signal for HER-2 is, in part, a function of many pre-analytical factors such as the nature and duration of fixation. Laboratories performing HER-2 IHC therefore require a system to address the possibility that pre-analytical factors may yield false negatives or positives. Before identifying such factors, however, laboratories need to know if the rates of HER-2 positivity at their individual institution fall within the expected range of nationally-determined values.

Design: We designed a two tiered statistical analysis to determine if the distributions of 2+ and 3+ positive HER-2 cases at 65 individual laboratories were statistically different from the overall distribution of 2+ and 3+ positive HER-2 cases derived from a total of 1738 cases analyzed during July-December 2003. For the 31 laboratories submitting more than 10 cases, we computed a standard chi-square statistic based on the squared differences between observed and theoretical (population) frequencies. For the 34 laboratories submitting 10 or fewer cases, we used a nonparametric randomization test consistent with small sample statistics. In both cases, we used 0.05 and 0.01 significance levels. For larger samples (i.e., $N > 20$), we also aggregated the data into two classes (0, 1) and (2+, 3+) and computed 95 percent confidence limits based on the binomial distribution to verify results.

Results: We analyzed 1738 breast cancer cases submitted from 65 institutions, using the two tiered statistical method. Of the 65 institutions, 1/31 failed the chi-square test, and 4/34 failed the randomization test. Further investigation of the individual cases from the institution failing the chi-squared test revealed that the rate of 2+, but not 3+, cases was abnormally low, and the number of 0 and 1+ cases abnormally high.

Conclusions: A two tiered statistical analysis-based quality assurance program can be used to monitor the rate of HER-2 positivity by IHC among different laboratories, and identify those whose positivity rates fall out of the expected range. Once identified, further studies can then be performed to identify pre-analytical or other factors which might account for the out-of-range results.

1501 Pulmonary Cytopathology Review: Experience at a Tertiary Care Hospital

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Background: Diagnostic accuracy in pulmonary cytopathology may be affected by errors in either identification and/or interpretation. The objective of this study was to determine if review of pulmonary cytologies by a second cytopathologist resulted in error reduction.

Design: Consecutive pulmonary cytology specimens, received over a three-month period at Henry Ford Hospital, were included in this prospective study. Each case was viewed by a cytotechnologist, a cytopathology fellow, and the primary cytopathologist, and then followed by blinded review by a second cytopathologist. Cases in which the two cytopathologists did not concur were reevaluated and a consensus diagnosis formulated. Concomitant or subsequent surgical biopsies were reviewed after generation of the consensus cytology diagnosis.

Results: Of the 112 cytology cases studied, 12 (12.7%) were unsatisfactory for evaluation, 46 (41.1%) were negative, 13 (11.6%) were atypical/suspicious, and 41 (36.6%) were malignant. There was cytologic concordance in 99 (88.4%). Of the 13 (11.6%) non-concordant cytologies, either the primary or reviewer cytopathologist had an atypical or suspicious diagnosis in 10 (76.9%) cases. 3 (23.1%) cases were benign versus unsatisfactory. In all atypical/suspicious cases, both cytopathologists identified an abnormality, but differed in the interpretation. 44 (39.3%) cytologies had surgical biopsies; 37 (84.1%) had concordant cytologies. 5 cases were negative on cytology, but had a malignant biopsy. 7 of the 13 (53.8%) non-concordant cases had a biopsy; 6 were malignant. Review of these 6 cases revealed that the primary cytopathologist gave an atypical/suspicious diagnosis in 4 (66.7%) cases, the reviewer cytopathologist in 1 (16.7%) case, and the subsequent consensus diagnosis was atypical/suspicious in 5 (83.3%) cases.

Conclusions: 1. Atypical/suspicious diagnoses in pulmonary cytology are less likely to have concordance between cytopathologists, and this likely reflects differing interpretations. 2. In atypical/suspicious cases, the consensus diagnosis tended to gravitate towards the center (atypical/suspicious diagnosis). 3. Although diagnostic agreement is less likely in atypical/suspicious cases, these cases may benefit from a review by another cytopathologist. 4. Routine review of all pulmonary cytologies is not recommended.

1502 Use of Tissue Microarrays for Clinical Assessment of Breast Cancer Biomarkers

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Background: Tissue microarrays (TMAs) are being increasingly used for cancer biomarker testing, providing equivalent or superior results in comparison to full section data. We have used TMAs successfully for QA purposes in diagnostic laboratories and have developed software tools for analyzing the data generated. To date, this technology has not been used for front-line assessment of biomarkers in the clinical setting. Here we describe a pilot study to assess the feasibility and cost-effectiveness of a centralized breast cancer biomarker program using TMAs for the province of British Columbia (BC).

Design: TMAs were constructed using triplicate 0.6, 1.0 and 1.5 mm cores from 88 breast cancer paraffin blocks (9 cores from each case). Sections from each TMA block were stained within 24 hours for ER and Her2 at two IHC laboratories using different automated stainers. TMA sections were scanned onto a BLISS server and scored by a pathologist. TMA results were compared to the original full-section data to determine the optimal number of cores and core size for clinical tissue microarrays.

Results: Compared to the 1.0 and 1.5 mm core arrays, the 0.6 mm core TMA had minimum core dropout with tumor cells observed in at least one core in all cases (87.5% of cases containing tumor cells in all 3 cores, 8% in 2 and 4.5% in 1). For Her2 complete concordance was observed amongst the 3 cores of all cases. One case showed a major disparity between the TMA results (3+) and the original full section (0). Gene amplification was present by FISH (ratio = 5.3) and repeat immunostaining on the full section revealed 2+ positivity. Greater variation between cores of the same case was observed with the ER staining. When the ER score from the strongest staining core was used, there was good correlation between the TMA and full section results. In a single case the TMA score was 1+ and the full section 3+, but no ER-positive tumors were negative in the TMA.

Conclusions: Our pilot data indicate that TMA results from triplicate 0.6mm cores are likely equivalent, and potentially superior, to whole section data. This study is being expanded to 500 cases to assess performance equivalence and streamline logistics for program implementation. In BC we estimate that a centralized TMA-based program would reduce reagent costs by up to 50 fold and enable the rapid assessment of novel biomarkers. Increased diagnostic accuracy would also be expected due to the inherent internal controls, elimination of inter-laboratory technical variation and reduced observer-variation.

1503 A Simple Modification of CAPChecklist Minimizes Extensive Editing and Reduces Clerical Error in Pathology Tumor Reporting

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Background: The American College of Surgeons Commission on Cancer (CoC) mandates that pathology reports at CoC-approved cancer programs include all scientifically validated or regularly used data elements for each site and specimen. In response to the requirement, the College of American Pathologists (CAP) has produced cancer protocols with checklists as a resource to pathologists. In order to be inclusive, the CAP checklists are often several pages long. After much editing, the final report rarely exceeds one page in most cases. This extensive editing often is cumbersome and makes room for clerical errors.

We designed a simple modification to the CAP checklists which minimizes the long list-checking and extensive editing (deletions), markedly simplifying routine pathology tumor reporting.

Design: The checklists from CAP protocols are used as the primary source. Each checklist is split into multiple much shorter sub-checklists specific for particular individual tumor types and stages of the same specimen type. Each of these sub-checklists is given a unique code using TMN designation, combined with a prefix to specify the specimen (see example below). The modified checklists and their unique codes are incorporated into existing electronic pathology reporting system (e.g. Cerner and Co-Path.).

Results: Entering a code brings a short checklist specific for a particular tumor type, at a specific TMN stage. For example, "CoAT3N1" brings a sub-checklist containing only the information pertinent to invasive colonic adenocarcinoma at T3 with regional lymph node metastasis. Blank spaces for tumor size, numbers of lymph node can then be filled out. Reviewing the long list of unrelated items or extensive editing is thus no longer necessary.

One can also type in a partial code or an organ name to pull up a list of the codes and titles of the sub-checklists pertinent to a specific organ. This modification can also be implemented in Microsoft Word text format or a simple database from which the final report can be copied and pasted into the hospital reporting system. Examples of the simplified checklists are available at:

http://pathology.uth.tmc.edu/faculty/bios/qu/CAPsynop.asp

Conclusions: This simple user-friendly modification of the CAP checklist reduces clerical error in Pathology tumor reporting by minimizing extensive editing and also enhances protocol compliance.

1504 Immunohistochemical Analysis of Estrogen (ER) and Progesterone Receptor (PR) Status in Breast Carcinoma (BC): Ten Year Cumulative Experience of the College of American Pathologist (CAP) Cell Markers Committee (CMC) Survey

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Background: ER and PR status of a primary BC is predictive of response to tamoxifen therapy. ER and PR analysis by immunohistochemistry (IHC) in formalin-fixed, paraffin embedded (FFPE) tissue is standard of care in the pathologic evaluation of BC. Results of CAP surveys over a ten year period illustrate the performance of a large cohort of IHC laboratories.

Design: For each survey, participants were sent unstained FFPE sections from the same invasive ductal BC shown by referee laboratories to be either ER/PR-positive or ER/PR-negative. Participants reported their qualitative interpretation of each survey case regarding expression of ER (+ or -) and PR (+ or -). For positive cases, participants graded immunoreactivity as follows: nonimmunoreactive, 1+ (<25% tumor cells positive, tcp), 2+ (26-50% tcp), 3+(51-75% tcp) and 4+ (>75% tcp).

Results: From 1994 to 2003, 11 survey cases (6 ER/PR negative, 5 ER/PR positive) were sent to participants. The number of participants increased from 133 to 575. For negative cases, the ability to correctly identify BC as ER/PR negative improved over time (P<.001,Cochran-Armitage trend test). Agreement with the reference score was higher with dichotomous (+/-) scoring for ER/PR (96.4%/85.6%) as opposed to graded scoring (72.6%/71.7%). Using +/- scoring, PR negative survey cases were correctly identified (95%) more often than positive survey cases (72%). For PR positive cases, the ability to identify samples differed within grades, with 4+ survey cases tending to be correctly identified more often than lower grade positive samples (Chi-Square test).

Conclusions: ER/PR negative BC cases were correctly identified more often than positive cases and the ability to correctly identify negative cases improved over time. The explanation for this observation is unknown but is likely related to technical improvements. Concordance was high with a positive or negative scoring but declined when semiquantitative grading was used. Technical variations may limit the ability for widespread accurate quantitation in an inherently qualitative test. Continued efforts at standardization are needed.

1505 A Robust System for Evaluating the Specificity and Performance of Antibodies Raised Against hTERT, the Catalytic Subunit of Human Telomerase

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Background: Telomerase enzymatic activity is closely correlated with human cancers and has potential diagnostic and prognostic utility. However, activity assays require fresh or frozen tissue lysates, give no information on telomerase expression at the single cell level or on subcellular localization, and specificity may be compromised by contaminating normal cells, such as activated lymphocytes, that possess intrinsic activity. For these reasons it is desirable to have well-characterized, specific antibodies against telomerase for research and clinical applications. Unfortunately, results published to date using a variety of commercially available anti-hTERT antibodies have been mixed. To remedy this, we developed a set of rigorous negative and positive control cells for use in evaluating the performance of anti-hTERT antibodies.

Design: Mouse embryo fibroblasts (MEF) were obtained from double knockouts for mouse telomerase (a gift from Dr. Lea Harrington, Univ. Toronto). Lacking telomerase, these cells represent excellent negative controls. KO MEFs were transfected with an HA-tagged hTERT/GFP expression vector providing isogenic positive control cells. Staining of putative anti-hTERT antibodies was assessed on these cells, as well as human cell lines known to either possess or lack telomerase activity.

Results: Transfetced MEF KO cells were easily detected using either GFP fluorescence or anti-GFP antibody. Anti-HA antibody confirmed telomerase expression in these cells. Transfected and untransfected cells grown on multi-chamber slides or fixed, paraffin embedded, and sectioned, allow convenient testing of antibodies. Of four commercially available antibodies thus far tested, none showed convincing, specific staining for hTERT. One widely used antibody displayed intense nucleolar staining in many human cell types, including some thought to be strictly telomerase negative. We did not observe any hTERT-specific staining in hTERT-transfected MEFs with this antibody even at very low dilution.

Conclusions: We have engineered mammalian cells strictly positive or negative for human telomerase for use in testing the specificity and performance of anti-telomerase antibodies. Use of these reagents should allow unambiguous assessment of putative anti-hTERT antibodies. Results to date using four commercially available antibodies fail to support specific hTERT staining for these antibodies.

1506 Validation of an Error Taxonomy System for Anatomy Pathology

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Background: Classifications of error in anatomic pathology (AP) have neither been tested for their ability to capture the full range of error in this domain nor tested for reproducibility among observers. In this study, we have developed a classification system to identify and capture all errors leading to amended reports, in a non-ambigious taxonomy.

Design: An error classification checklist was developed and tested for its validity by two observers examining amended reports of three consecutive years (2001–2003) from Henry Ford Hospital, Anatomic Pathology, generated from roughly 150,000 surgical pathology cases. Defects were evaluated in the three main stages of testing. Pre-analytic stage errors included those of patient or tissue identification, defects in specimens, gross description, erroneous measurement or inadequate representativeness. Analytic stage errors included defective interpretation, classified as "overcall" or "undercall" in diagnosis and misclassifications or refined diagnoses. Post-analytic stage errors related to defective reports containing nondiagnostic information errors, typographical or computer format errors. Simple kappa values were calculated to assess interobserver variablity in classification.

Results: Using this tool there was a fairly consistent amended report frequency of 2.3-3.3 amended reports per thousand case accessions. Diagnostic mis-interpretations were not the most common errors and accounted for a narrow range of 23-28% of the amended reports. Defective specimens were the least common error, 4-10%. The most common error type was a defective report, 28-44%. The second most common error was that of wrong identification, 27-38%. There is good interobserver agreement in the identification and classification of the error types in this validation study with 91.4% agreement among the two observers (kappa value 0.8780).

Conclusions: These data are derived from the assessment of surgical pathology amended reports using a standarized and validated assessment tool. This information will allow insight into process defects leading to error, so that we may better understand true outcomes of error in anatomic pathology and how to minimize adverse events by testing novel interventions and system redesign.

1507 Pathology Panel Review of Breast Lesions: A Quality Assurance Assessment

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Background: Assessment of benign and malignant breast lesions can be difficult, particularly on needle biopsy. False positive and negative diagnoses can result in potentially unwarranted over and under treatment with subsequent adverse medicolegal consequences. Assessment of breast lesions using a breast pathology panel has been advocated to address quality assurance and quality improvement issues. The aim of this study, therefore, is to evaluate the effectiveness of a breast pathology panel double review in the assessment of benign and malignant breast lesions.

Design: Baseline (pre-intervention) evaluation (voluntary intradepartmental consults) of all breast specimens received at the Henry Ford Health System (including needle biopsy, localizations, mastectomy specimens, sentinal and axillary lymph nodes) from Jan 2002- July 2003 (surgical pathology volume=78,000). Error verification sources included clinician and tumor board review. Intervention included prospective double slide review of all breast specimens facilitated by a Breast Review Panel comprised of 4 pathologists with an interest in breast pathology (including one specialist breast surgical pathologist) from Aug 2003-June 2004 (surgical pathology volume =36,000). All slides from each case were reviewed by at least one of the panel pathologists. All tumor cases were reviewed by the panel pathologist involved in tumor board presentation (in most cases the specialist breast surgical pathologist). Key slides from all breast cases (benign and malignant) were reviewed by the panel pathologists as a group at daily slide review.

Results: Over the baseline period a total 37 revised diagnoses were rendered overall of which 5 (13.5%) were revised breast diagnoses. During the intervention (breast review panel) assessment period a total 18 revised diagnoses were rendered with no breast diagnosis revisions (0%). The only corrections to breast specimen reports during the intervention period were for typographical errors (2 stage, 1 margin and 1 side designation change).

Conclusions: This study shows that a Breast Review Panel facilitated double review of breast specimens has the potential to minimize erroneous breast cancer diagnoses prior to clinician/tumor board review with subsequent reduction in potential patient over treatment and adverse medicolegal consequences. This approach may have merit in some practice settings as an effective patient safety initiative.

1508 Measuring Patient Preferences for Ancillary Testing: Patient Willingness-To-Pay for Immunohistochemistry in Tumors of Unknown Origin

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Background: Previous studies have shown that patient outcomes are better when physician-patient shared decision-making takes place; however, patient preferences regarding pathology diagnostic testing have rarely been measured. Patient preferences regarding pathology ancillary testing for tumor identification have never been measured.

Design: A contingent valuation (willingness-to-pay [WTP]) survey was administered to 45 volunteer patients presenting to an obstetrics and gynecology outpatient clinic.routine Patients were asked to imagine having *either* a metastatic cancer of unknown origin (MCUO) *or* a previously diagnosed and treated breast cancer with a newly identified malignancy (BCNM). After receiving additional information about immunohistochemistry (IHC) and its diagnostic performance, patients were asked to indicate how much they would be willing to *pay out of pocket* for IHC analysis of their tumor in an attempt to identify its origin, under conditions when tumor identification might make a difference in his/her survival and also when it would not. Volunteers were asked to provide demographic, household income, quality of life (QOL), and cancer history information in order to examine factors that may influence WTP.

Results: The mean age of the respondents was 37 years old and most had one child. The majority had annual household incomes between $35,000 and $99,999 and judged their current QOL to be good (mean rating of 9 out of 10). For both MCUO and BCNM scenarios, respondents were WTP an average of $1900 for IHC testing. Respondents were WTP this amount for IHC testing even if it did not affect their clinical outcome but

allowed them to know the origin of their tumor. Respondents were WTP more for IHC testing when its diagnostic performance was assumed to be high. Preliminary statistical analyses examining factors linearly correlated or significantly associated with WTP did not reveal any factors clearly driving respondents' WTP.
Conclusions: In this study examining patient preferences for anatomic pathology ancillary testing, patients reported being WTP approximately $2000 for the information IHC may provide regarding tumor origin, even if this information didn't improve their long-term clincial outcome. Depending on the particular IHC work-up utilized, IHC appears to be cost beneficial in the work-up of these two groups of cancer patients.

1509 Improving Patient Safety by Examining Pathology Errors: Year 2 Findings

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Background: Four institutions are currently sharing anatomic pathology error data collected for a 5-year grant sponsored by the Agency for Healthcare Research and Quality. This report details the frequency of error and the successes and failures of specific error reduction plans.
Design: Six years of cytologic-histologic correlation data have been entered into a web-based database, and these data have been used to create error reduction plans. Data collected include specimen site, type of error (sampling or interpretation), severity of error, and specific pathologist or cytotechnologist provider. Error frequencies and interobserver variability were calculated by each institution examining errors detected at other sites. Error reduction plans included the introduction of double viewing pulmonary cytology specimens, creation of specific sign-out terms for gynecologic histologic specimens, and review of thyroid aspiration specimens to determine error cause. Success of error reduction plans was monitored by determination if error reduction plans reduced errors.
Results: Frequency of error per institution for gynecologic specimens ranged from 1.7% to 9.5% of all cases correlated. Frequency of error per institution for non-gynecologic specimens ranged from 5.9% to 11.7% of all cases correlated. Pairwise institutional kappa values for agreement on error type (sampling or interpretation) ranged from 0.12 to 0.74. Some institutions thought that none of their errors resulted in patient harm whereas other institutions thought that 30% of errors resulted in patient harm. Error reduction plans had variable effect on decreasing error frequencies. Double viewing pulmonary cytology specimens had no effect on decreasing errors whereas targeting thyroid aspiration resulted in decreasing false negatives.
Conclusions: Errors occur at a high frequency and there is little institutional agreement regarding error cause and error severity. Error reduction plans have met variable success and are highly dependent on local culture. Error reduction plans designed to target specimen quality have achieved greater success than plans designed at decreasing interpretive error.

1510 Error Reduction in Specimen Labeling

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Background: Specimen labeling errors that occur during accessioning can result in significant adverse and sentinel events. In mid-2003, our error rate had escalated to weekly occurrences. Among the most serious consequences included two separate incidents where pap smear identification was unable to be verified and 4 patients had to be called back to be re-sampled. Another incident involving a breast core biopsy was only discovered when the histologic findings did not correlate with the mammogram impression. Following these, a QA group was organized to examine the process.
Design: The major "Toyota" principles we employed were 1. No batching 2. utilize a "pull" system rather than a "push" system 3. Ensure a perfect product at hand-offs. Our specimen handling process was mapped on a blackboard beginning with the final product (a correctly labeled slide). Each step in the process was examined ending with the receipt of the specimen. Contributing factors were also examined. Redundancies (opportunities for error) in the system became apparent with this backwards or "pull" approach. All changes implemented were considered temporary until verification.
Results: It was apparent that batching at the receiving area was a probable source of error. Contributing factors included the frequency of the task, similar patient names, similar specimen types and frequent work interuptions. Labeling specimens singly at the time of accession greatly reduced the chance of adhering the wrong label. Confusion between similar specimens and names was eliminated by working with just one specimen until completion. Accessioners were sharing a label printer which required walking to the shared printer to retrieve ones own labels. In addition to the elimination of batching, we invested in separate label printers for all accessioners.
Conclusions: Initially there was some staff resistance to the new no batching policy due to perceptions that batching was more efficient. However, after a 12 month period, labeling errors have dropped from 1-2 per week to 2 in 12 months. Both of these errors were caused by new trainees batching their work. We plan to apply the principle of no batching to other areas within anatomic pathology in the future.

Techniques

1511 Mutational Analysis of *CEBPA* in Acute Myeloid Leukemia: An Integrated Approach Using Denaturing and Non-Denaturing Capillary Electrophoresis and Direct Sequencing

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Background: C/EBP$^{\alpha}$ (CCAAT/enhancer binding protein-alpha) is a transcription factor that regulates myeloid differentiation. Mutations in *CEBPA* are present in approximately 10-15% of intermediate risk AML and were shown to be a favorable prognostic factor. The variety of mutations that occur in *CEBPA* make rapid definitive assessment of mutational status difficult.
Design: We developed a mutational analysis method for *CEBPA* using capillary electrophoresis (CE) under denaturing (GeneScan sizing) and non-denaturing (single strand conformational polymorphism-SSCP) conditions. Genomic DNA was extracted from the bone marrow or blood of 40 patients with AML (FAB: 3-M0; 10-M1; 16-M2; 9-M4; 1-M7; 1-MDS). The N-terminal (550 bases) and C-terminal (680 bases) regions of *CEBPA* were amplified in separate PCR reactions, using FAM-labeled forward primers and HEX-labeled reverse primers. PCR fragments were analyzed by CE and confirmation of the detected mutations was done by direct sequencing.
Results: SSCP/CE analysis detected eight (20%) N-terminal fragments and ten (25%) C-terminal fragments with mobility shifts as compared to wild-type. Sequencing analysis revealed six (15%) samples with N-terminal mutations (four single base insertions and two single base deletions) and ten (25%) samples with C-terminal sequence changes (five polymorphisms and five changes, involving deletions, insertions and duplications of various number of bases). Comparison of SSCP with sequence analysis revealed that 16 of 18 samples with mobility shifts had sequence variations. Careful review of the two discrepant cases by direct sequencing revealed sequence variations in only one strand. The remaining samples without mobility shifts in SSCP/CE analysis were also unmutated by sequence analysis. Fragment sizing by Genescan was useful in determining the number of bases involved in sequence variations larger than two bases.
Conclusions: These results indicate that the use SSCP/CE in tandem with GeneScan fragment size analysis are reliable and rapid initial screening methods for mutation detection in *CEBPA*. Despite the wide spectrum of mutations that occur in *CEBPA* in AML patients, there was a high concordance rate between direct sequencing and SSCP. Therefore, this approach will reduce the time and money spent on mutation detection using direct sequencing by at least 70%.

1512 High Sensitivity and Specificity of FISH in Routine Detection of t(14;18)(q32;q21) and t(11;14)(q13;q32) in Formalin-Fixed Paraffin-Embedded (FFPE) Tissue Sections Using Automated Morphometric Image Analysis

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Background: PCR detection of the t(14;18)(q32;q21) and t(11;14)(q13;q32) has remained the primary method for confirming the presence of chromosomal translocations characteristic of follicular (FL) and mantle cell lymphomas (MCL), respectively. However, the sensitivity of these PCR-based assays is suboptimal in FFPE tissues with reported rates of ~60-75% for t(14;18) and ~30-40% for t(11;14). We assessed the sensitivity and specificity of 2-color interphase FISH for the detection of t(14;18) and t(11;14) in archival FFPE tissue sections using morphometric image analysis.
Design: All lymphoid tissues were examined morphologically and fully characterized by immunohistochemistry. All MCLs showed nuclear cyclinD1 protein expression. Deparaffinized tissue sections were incubated with Vysis probes (IgH gene;14q32;Spectrum Green and either bcl-2 gene;18q21;Spectrum Orange or cyclinD1 gene;11q13;Spectrum Orange) following digestion/pretreatment. Morphometric analysis was performed using a MetaSystems™ imaging system with extended focus/tile sampling methodology. Signals in close proximity (<2 pixels) were considered positive and the percentage of positive tiles was calculated for each case. Case positivity was defined as 3 standard deviations above the mean of a translocation-negative group.
Results: In t(14;18)(q32;q21) FISH assays, 31 of 34 FLs showed a t(14;18), whereas none of the reactive lymph nodes (n=7) or non-follicular, B-cell lymphomas (n=23; including 2 CLL/SLLs, 6 MZBCLs, 11 MCLs) contained a t(14;18). The sensitivity and specificity of the t(14;18) FISH assay was 91.2% and 100%, respectively. A t(14;18) could be demonstrated in 2 FLs undergoing histologic transformation to large B-cell lymphoma and in a single FL showing marginal zone differentiation. 1 of 7 de novo DLBCLs showed a t(14;18) by FISH. In t(11;14)(q13;q32) FISH assays, 21 of 21 MCLs contained a t(11;14), whereas none of the reactive lymph nodes (n=10) or non-mantle cell, B-cell lymphomas (n=16; 2 CLL/SLLs, 3 DLBCLs, 11 FLs) contained a t(11;14). Both the sensitivity and specificity of the t(11;14) FISH assay were 100%.
Conclusions: 2-color interphase FISH is a rapid, highly sensitive and specific method for the routine detection of t(14;18) and t(11;14) in archival FFPE tissues using automated morphometric analysis and offers a significantly higher rate of detection than that reported for standard PCR.