Conclusions: The patients with solid or MPP subtypes had a worse prognosis even if their subtypes were not predominant. Therefore we need to take care of solid or MPP subtypes at the time of diagnosis.

1941 Malignant Melanoma Presenting as Thoracic Midline Malignancy: Clinicopathological and Molecular Features

Keluo Yao, Weiqiang Zhao, David Cohen, Alejandro Gru, Greg Otterson, Konstantin Shilo. The Ohio State University Wexner Medical Center, Columbus, OH.

Background: Malignant melanoma (MM) typically occurs in the skin, but also may occur in mucosal membranes and, rarely, visceral organs. Approximately 30-40% of cutaneous MM harbor activating BRAF V600 mutations providing the basis for treatment with BRAF kinase inhibitors. Primary thoracic MM is exremely rare with very few cases reported in the literature and their molecular profile is largely undefined. The aim of this study was to detail clinicopathological and molecular findings of MM in this uncommon location.

Design: Based on review of surgical pathology reports from January 2012 to June 2015, five cases of malignant melanoma presenting as a thoracic midline lesion were identified. The histological diagnosis was confirmed on subsequent re-review of H&E and immunohistochemical stains. Sanger and/or Next Generation Sequencing were employed for molecular profiling. Review of electronic medical records revealed no evidence of an extrathoracic primary.

Results: The tumors occurred in 5 Caucasian patients (4 males and 1 female, medium age of 71.6 years, range from 54-80). The patients had site specific symptoms including dyspnea (2/5), cough (2/5), hemoptysis (1/5), and change in voice (1/5). At presentation, imaging studies showed disease confined to the chest (5 of 5), including middle mediastinum (4 of 5) and lung hilum (3/5). At light microscopy, 2 of 5 tumors featured cytoplasmic melanin. All five tumors showed expression of melanocytic markers. Four of 5 patients died of the disease within one year (mean follow up time five months). One tumor harbored BRAF V600K mutation and showed partial response to BRAF inhibitor. None of 4 tested cases showed BRAF V600E mutation.

Conclusions: MM presenting as a thoracic midline mass lesion elicits site specific symptoms likely due to central location associated with the injury to large airways. While their light microscopic morphology and immunohistochemical profile are identical to cutaneous MM, our analysis documents that MM in this location shows an aggressive clinical course and potentially different spectrum of BRAF mutations.

1942 Patchy High Frequency of Nonspecific Abnormal Signals (Background Noise): A Common Pitfall in Interpretation of ALK FISH Results

Hong Yin, Shaobo Zhu, Michele K Zelonis, Dana R Snyder, Zongming E Chen. Geisinger Medical Center, Danville, PA.

Background: The presence of EML4-ALK fusion as a consequence of ALK gene rearrangement is a useful biomarker to predict therapeutic responsiveness to anti-ALK agent in a subset of non-small cell lung cancer (NSCLC). The gold standard assay for detection of ALK rearrangement is by FISH analysis. Interpretation of FISH results can be difficult in some challenging cases. Patchy high frequency of abnormal signals is recognized as one potential pitfall that may lead to false positive results. Here, we report the frequency, distribution pattern, and useful remedy for this challenging diagnostic issue based on our own experience in Geisinger Medical Laboratory.

Design: A total of 188 FISH analysis for ALK gene rearrangement to investigate the background noise. In addition, there were 17 positive samples from our validation included in this study in order to compare the signal patterns. Cases with high background noise (abnormal signals > 10%, falsely positive) were identified. FISH analysis was repeated using different specimens from the same patients. The signal patterns were carefully analyzed by two and more specially trained FISH technologists and pathologists. The characteristics of background noise signals were compared with true positive signals.

Results: There were 3 of the 188 (1.6%) cases with ALK gene rearrangement. A total of 20 ALK gene rearrangement samples were studied. In these 20 samples, the positive split signals showed a diffuse abnormal pattern. Non-tumor areas were compared, although abnormal signals can occasionally be seen, it is usually patchy and focal.

There are 5 cases that reveal high background noise, 3 cases up to 16-18% positive signals. The distribution of background noise appears as abnormal split signals were focal, and not a true diffuse pattern. When carefully examined abnormal signals were also found in adjacent non-tumor cells when compared to areas containing tumor.

It would be interesting to confirm our hypothesis – background noise up to 18%. For this study, we repeated ALK FISH assay for two patients using different specimens. Upon repeating with different specimens, the background noise decreased to 2-6%. The third patient had tumor containing an EGFR mutation, essentially precluding any possible ALK gene arrangement.

Conclusions: Accurate assessment of ALK gene rearrangement by FISH is critical for identifying patients with NSCLC who may benefit from ALK inhibitor therapy. Our data indicate that it is important to recognize this background noise, which may be a potential pitfall in interpretation of ALK FISH results.

1943 Dysregulated miR-185-STIM1 Axis in Obliterative Bronchiolitis

Chen Zhang, Guang-Sheng Lei. Indiana University School of Medicine, Indianapolis, IN.

Background: Obliterative bronchiolitis (OB) is the leading cause of graft failure and death after lung transplantation. The diagnosis of OB can be challenging even among experienced pathologists. No effective biomarker or treatment for OB is available. Epithelial-to-mesenchymal transition (EMT) plays critical roles in the pathogenesis of OB. STIM1 (stromal interaction molecule 1), an endoplasmic reticulum Ca^{2+} sensor and a direct target of miR-185, has been shown to promote EMT in cancer cells. We hypothesized that the miR-185-STIM1 axis is dysregulated during OB.

Design: Fifteen patients who underwent second lung transplantation due to OB in our institution from 2005-2015 were included as our study objects. The sections of explant lung were examined microscopically and a block showing typical OB lesion was selected from each patient. Normal appearing lung tissue from age and sex matched group of 15 patients was used as control group. The study has been approved by the Institutional Review Boards. The STIM1 immunohistochemical stain was performed on a 4-mm microscopic slide cut from the above mentioned blocks. Total RNA was isolated from formalin-fixed paraffin-embedded (FFPE) lung tissue and was reverse transcribed into cDNA. The expression levels of miR-185 were analyzed by real-time PCR, and compared between the two groups using unpaired student's t-test.

Results: STIM1 was overexpressed in airway epithelium of the lungs with OB, compared with the normal airway epithelium that expressed no or low level of STIM1. The expression level of miR-185 was significantly decreased in lungs with OB compared with that in normal lungs ($P<0.0001$).

Conclusions: The EMT-related miR-185–STIM1 axis was dysregulated in the lungs with OB, with STIM1 overexpression and miR-185 down-regulation. The dysregulated miR-185-STIM1 axis may serve as a candidate biomarker for diagnosis and a target for therapies.

1944 Comparison of Mutational Profiles in Cytology and Corresponding Surgical Specimens from Patients with Lung Adenocarcinoma

Hamid Zia, Alan Marcus, Brian E Geraghty, Rana S Hoda, Navneet Narula, Hanna Rennert, Helen Fernandes. New York Presbyterian Hospital-Weill Cornell Medical College, New York, NY.

Background: Recent data suggests that surgical specimens from patients with Lung Adenocarcinoma produce a more consistently viable tumor sample for molecular analyses, than cytology cell blocks. Minimal tumor material available in cytology cell blocks is often a limiting factor for multigene molecular assays. We compared mutational profile of cytology and corresponding surgical specimens from patients with Lung Adenocarcinomas.

Design: 16 patients (8 female, 8 male; mean age 68 years) with a diagnosis of Lung Adenocarcinoma on cytology (cell blocks) and surgical specimens from same anatomic site were tested. DNA was extracted from the selected tumor tissue and subjected either to targeted Next Generation Sequencing (NGS) using the 50-gene hotspot panel or a realtime PCR assay for detection of *EGFR* and *KRAS* variants. A two tailed student t-test was used for statistical analysis.

Results: A specific diagnosis of lung adenocarcinoma was rendered in 83% of the FNA cases and immunostains for TTF-1 were positive in these cases. The average percentage of neoplastic cells and extracted DNA concentration for cytology specimens was significantly lower than the surgical specimens [Neoplastic cells: 31% vs 68% (*p< 0.0001); DNA concentration: 5.5 ng/ul vs 58.8 ng/ul (**p=0.025). 6 *KRAS* and 4 sensitizing *EGFR* variants were detected in cytology and corresponding surgical specimens from 10 patients. 5 cases were negative for variants in both specimen types. One surgical specimen had a *KRAS* Q61H variant that was absent in the corresponding cytology specimen. This discordant specimen had two surgical blocks tested, with the other block giving an identical result to cytology. 6 additional variants (*TP53*=2; *PIK3CA*=1; *APC*=2; *STK11*=1) were identified in cytology; corresponding surgical specimens had 8 variants including *TP53*=2; *PIK3CA*=2; *APC*=2; *STK11*=1 and *CDKN2A*=1).

	Ave Neoplastic cells (%)	Ave DNA conc. (ng/uL)	# of *EGFR* variants	# of *KRAS* variants	Addt'l variants	Total # of variants
Cytology	31	5.5	4	6	6	16
Surgical	68*	58.8**	4	7	8	19

Overall there was 91% concordance rate among actionable variants and >85% correlation among all variants identified in both specimen types.

Conclusions: Despite significant differences in the percentage of neoplastic content and DNA concentration, the NGS results from cytology are comparable to surgical specimens. Cytology specimens are excellent candidates for interrogation of genomic targets with therapeutic significance in patients with lung adenocarcinoma.

Quality Assurance

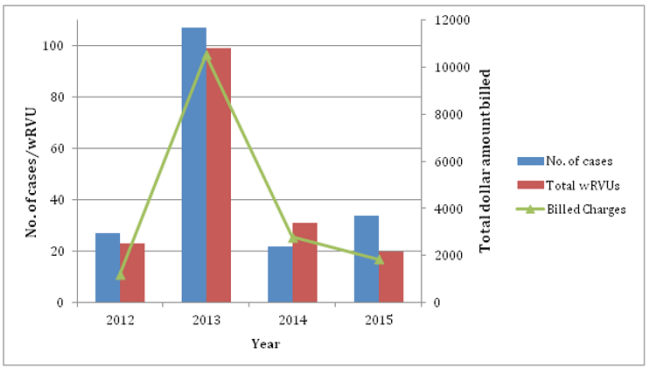
1945 Cost-Benefit Analysis of C4d Immunoflourescence and Immunohistochemistry in Evaluation of Pulmonary Allograft Dysfunction

Monica Aldulescu, Razvan Lapadat, Robyn Essendrop, Kamran M Mirza, Swati Mehrotra, Vijayalakshmi Ananthanarayanan. Loyola University Medical Center, Maywood, IL.

Background: Antibody-mediated rejection (AMR) is a well-recognized cause of allograft dysfunction in solid organ transplants of the kidney and heart, however no consensus definition exists on its occurence in lung transplantation. Detection of the specific complement degradation product C4d by immunofluorescence (IF) or immunohistochemistry (IHC) has shown to be a sensitive and specific marker for AMR; however, its role in evaluating pulmonary allograft rejection remains contentious. The aim of this study is to determine the frequency and clinical impact of C4d IF and IHC and to determine a cost-benefit analysis for performing these tests in lung allograft recipients.

Design: Cases of transbronchial lung transplant biopsies were identified using a retrospective review of our database from 2012 to 2015. A total of 194 cases in 104

patients were identified and evaluated with regards to C4d IF/IHC stain positivity and clinical significance. We also performed a retrospective review over the same time period to evaluate lung allograft recepients for donor-specific antibody (DSA) status.
Results: Of the 194 cases, 1 (0.5%) was interpreted as "positive," and 5 (2.5%) were interpreted as "weakly/focally positive." During this review period, only 1 out of a total of 8 patients who were clinically deemed as AMR with supporting DSA positivity, showed focal positivity for C4d by IHC. We also observed a sharp rise in C4d testing in 2013, with a subsquent decline and stabalization over the following two years. Finally, the total cost incurred (billed charges) and total work relative value units (wRVU) were calculated based on the number of cases per year with the results depicted in Figure 1.



Conclusions: C4d is a poor surrogate marker for antibody-mediated rejection in lung transplant patients. We can also conclude that while the dollar amount and wRVUs do not seem very expensive, in the big scheme of things, the results reflect a wastage of healthcare dollars. It may seem in the best interest of pathology practice to do stains in the era of RVU-based reimbursement; however the lack of correlation with DSA, predominantly negative or inconclusive, do not justify these tests in routine practice and should be abandoned.

1946 Ambiguous p53 Immunohistochemical Patterns and Role of Tissue Processing: Correlation with DNA Sequencing

Anand Annan, Joe Z Zhao, Kelly Smith, Sanam Husain, Elizabeth Gillies, Rosemary Zuna. University of Oklahoma Health Sciences Center, Oklahoma City, OK; OU Medical Center, Oklahoma City, OK.
Background: Immunohistochemical (IHC) staining of p53 is used as a surrogate marker for *TP53* mutation status, particularly in high grade serous carcinomas. Wild type, null and mutated staining patterns have been reported with various scoring systems to correlate with *TP53* mutation status. We evaluated ambiguous IHC patterns with heterogeneous staining intensity and correlated them with the *TP53* mutation status by Sanger sequencing and the role of tissue processing (cold ischemic time and formalin fixation time) in ambiguous IHC pattern.
Design: 22 endometrial carcinomas and 5 ovarian serous tumors showing various p53 IHC patterns (Clone Bp53-11 with ultraview Universal DAB kit using Ventana Benchmark XT stainer) were selected for Sanger DNA sequencing for exons 4-10 using thick sections from the formalin-fixed paraffin embedded (FFPE) tissue blocks. To evaluate the role of processing, fresh tissue from 5 ovarian tumors were divided into two cohorts (cold ischemic time of < 60 mins and >60 mins) and formalin fixed for varying intervals (6-24 hours, 24-48 hours, 48-72 hours, and >72 hours). Tissue sections from the resulting FFPE blocks were cut and immunostained for p53 as well. Fisher's exact test was performed to determine the statistical significance between groups.
Results: An IHC staining pattern of at least 50% (range: 55-100%) positive tumor cells (irrespective of staining intensity) correlated with missense mutation in 94% of cases (16/17) and staining pattern of < 50% (range: 7-42%) correlated with wild type pattern in 100% of cases (5/5) (p=0.002, Fisher's exact test). Null pattern (0% staining in tumor cells) correlated with frameshift mutation in 80% of cases (4/5). No mutation was identified in 1 of 5 cases with null pattern and in 6% (1/17) of cases with > 50% positive staining pattern. There was no significant difference in p53 IHC staining pattern related to tissue processing.
Conclusions: High expression of p53 (≥50% positive tumor cells) or null pattern were highly indicative of *TP53* mutation. Ambiguous staining patterns with heterogeneous stain intensity can be clarified by determining the percentage of positive cells, such that ≥50% =mutated p53 or <50%=wild type p53. Because of possible prognostic significance of p53 frame shift mutation, the recognition of null pattern is important and requires close attention to the wild type p53 pattern in the surrounding stroma. Variation in tissue processing had no significant effect on the p53 IHC pattern.

1947 Can Decalcification of Thyroid Tissue Cause Overdiagnosis of Papillary Thyroid Carcinoma?

Dhanalakshmi Balakrishnan, Chengbao Liu, Laura Birney, Syed Hoda, James Davis, Alan Heimann, Jingxuan Liu. State University of New York, Stony Brook, Stony Brook, NY.
Background: This study was based on an incidental finding at our institution - fresh benign thyroid tissue that had been placed directly into decalcification solution showed evidence of nuclear clearing microscopically. This finding was strikingly similar to the nuclear clearing characteristically present in papillary thyroid carcinoma (PTC). The diagnosis of PTC is based upon a cluster of features such as nuclear clearing, nuclear grooves, and nuclear inclusions. PTC can also cause extensive dystrophic calcifications in the thyroid gland. Due to the presence of calcifications in both benign and malignant thyroid tissue, decalcification of the specimen is often required. This study aims to examine the effects of decalcification on thyroid tissue and its potential ability to mimic PTC.
Design: To further explore our initial incidental finding, we examined 10 fresh thyroid specimens. The specimens were divided into two groups. In one group, we placed the fresh tissue in formalin for one hour, and then divided this tissue into three pieces. One piece was placed into slow decalcification solution for 1 hour, one piece for 4 hours and one piece for 15 hours. In the second group of ten, the tissue was again divided into three pieces. One piece was placed directly into decalcification solution without prior formalin fixation for 1 hour, one piece for 4 hours and one piece for 15 hours. The tissue was then submitted for histological processing.
Results: The 10 cases which were fixed in formalin for one hour prior to decalcification did not show evidence of nuclear clearing in the benign thyroid tissue. However, the 10 cases which were put in decalcification directly for 1, 4, and 15 hours all showed significant nuclear clearing, mimicking our initial incidental findings. Despite nuclear clearing, the other diagnostic features of PTC such as nuclear grooves, crowding and nuclear inclusions were absent.
Conclusions: Decalcification of fresh thyroid tissue without at least one hour of formalin fixation time can produce significant nuclear hypochromasia, thus mimicking the nuclear clearing seen in PTC. This can make accurate diagnosis not only confusing and difficult, but can ultimately have detrimental consequences. This effect can be prevented by placement of fresh tissue in formalin for at least one hour prior to placement in decalcification solution. This important finding can prevent false diagnoses of papillary thyroid carcinoma.

1948 Communication from Referring Clinicians to Pathologists in the Electronic Health Record Era

Andrea L Barbieri, Oluwole Fadare, Christopher Sebastian, Hardeep Singh, Vinita Parkash. Yale University, New Haven, CT; University of California San Diego, La Jolla, CA; VA Health Services Research Center for Innovation & Baylor College of Medicine, Houston, TX.
Background: Electronic health records (EHRs) change the way physicians communicate. In this exploratory study, we sought to determine changes in patterns of communication between clinicians and pathologists with EHR implementation, especially in regard to missed information and its potential for patient harm.
Design: Most EHRs offer message inboxes to facilitate communications between clinicians. We examined EHR mail communications of pathologists at three institutions, two within the same health system to analyze type, quality and response time to communication. The APLIS was queried to assess if requests were responded to. Pathologists and clinicians answered a brief questionnaire on the use of electronic communication through the EHR.
Results: All three institutions used the same EHR vendor. One institution had EHR mail inactivated for pathologists. Of 17 pathologists queried at the remaining 2 facilities, 5 had never accessed the EHR under their own name. Another 4 were unaware of the existence of EHR mail. Assessment of the communications in the mailboxes of the remaining 12 pathologists indicated pending messages ranging between 0 and 40. Of 20 communication items assessed, 3 alerted pathologists to errors in the pathology report. In 6 cases requests for follow-up studies apparently went unanswered by pathologists (range 3 weeks to 4 months). In 2, requests were made not directed to the primary reporting pathologist but a subspecialty pathologist who the clinician was familiar with. In the remaining cases, clarification of reports was requested and/or follow-up information was provided. Response through other communication means could not be assessed. Clinicians assumed that pathologists routinely checked their messages in the EHR because that was their preferred mode of communication with other physicians on patient issues. Interestingly, at the institution where the mail function was turned off for pathologists, support personnel maintained access and handle requests for follow up testing using this modality.
Conclusions: There appears to be a communication gap between primary clinicians and pathologists in the EHR era. Pathologists are unaware of EHR-based communication applications and rarely, if ever, review their inboxes. As we become increasingly reliant on electronic communication, pathologists must participate as clinical team members to ensure reliable communication with their clinician counterparts.

1949 Differences in Ki67 Percent Identified during Breast Consult Review Do Not Impact the IHC4 Score: A Quality Assurance Study

Gonzalo Barraza, Erinn Downs-Kelly, Rachel E Factor. University of Utah Health Sciences Center, Salt Lake City, UT.
Background: A number of studies have shown that standard histopathologic variables with ER, PgR, HER2 and Ki67 can be included in equations (Magee Equation3, IHC4 score) that correlate with the Oncotype Dx® recurrence score. While the first three markers are well regulated by ASCO/CAP, there are, as yet, no guidelines for how to quantitate and report Ki67. Despite this, and despite the value of IHC4 score only in research at this time, Ki67 and IHC4 are being reported by pathologists at community hospitals, and are utilized by some clinicians in making treatment decisions. At our academic institution, we review pathology cases from outside hospitals (OSH) when patients relocate their care. For this study, we asked how often Ki67 differed upon review of breast cases, and whether this impacted the reported IHC4 score.
Design: A database search of breast pathology consults was conducted at the University of Utah from 2013 to 2015. Hospitals that reported ER, PgR, HER2 and Ki67 and sent slides for review were included. These hospitals reported an IHC4 score based on Magee Equation 3, which can be found online. We recorded the Ki67, ancillary markers and the IHC4 score reported by the OSH. We recorded the Ki67 from our

review (semiquantitative without imaging software) and re-calculated the IHC4 using our results. We recorded if the Ki67 was estimated using manual or digital imaging techniques at the OSH.

Results: Our search found 71 OSH breast pathology cases that fit criteria. 80% of the OSH cases used digital imaging (Aperio) to estimate Ki67. IHC4 reporting was based on Magee Equation 3. The hospitals reporting IHC4 qualify the results with the Onctoype Dx scale (<18=low, 18-30=high, >30=high). There were no significant differences in ER, PR and HER2 results from the OSH and the consults. When comparing Ki67 reported from the OSH and our interpretation, 49% (35/71) showed differences in Ki67 values of 5-10 percentage points. 27% (19/71) showed differences in Ki67 values greater than 10 percentage points. Despite these differences, only two cases (2.8%) had an IHC4 value that changed category (both were initially described as intermediate, and were re-calculated as low).

Conclusions: Though IHC4 is not routine, it is included in some pathology reports and clinicians are utilizing this ancillary information in managing patients. This study demonstrates that differences in Ki67do not appear to significantly impact IHC4 results.

1950 Results of College of American Pathologists Proficiency Testing for Highly Sensitive ALK Immunohistochemistry in Lung Adenocarcinoma

Andrew M Bellizzi, Robert A Schwartz, Jason L Hornick, CAP Immunohistochemistry Committee. University of Iowa, Iowa City, IA; Manchester Memorial, Manchester, CT; Brigham and Women's, Boston, MA.

Background: Lung adenocarcinoma (AdCA) is characterized by recurrent molecular genetic alterations, many of which are clinically actionable. One of these is *ALK* translocation, resulting in constitutive tyrosine kinase (TK) activation. *ALK*-rearranged lung AdCA may respond dramatically to the TK inhibitor crizotinib. The CAP/IASLC/AMP Lung Cancer Molecular Testing Guideline recommends ALK testing in advanced lung AdCA. Fluorescence in situ hybridization (FISH) is typically utilized, although several groups have reported promising results with highly sensitive ALK immunohistochemistry (IHC). In addition to typical advantages of IHC relative to FISH (e.g., cost, turnaround time, ease of interpretation), there are reports of ALK IHC-positive/FISH-negative tumors responding to crizotinib. The ALK fusion protein is expressed at low-levels in lung AdCA, and the commonly used ALK1 clone has been shown to perform suboptimally in this setting. Herein, we report results of initial CAP proficiency testing for highly sensitive ALK IHC.

Design: Participating laboratories received an unstained tissue microarray slide containing cores of 4 (FISH-confirmed) *ALK*-rearranged lung AdCAs, 5 *ALK*-non-rearranged lung AdCAs, and 1 anaplastic large cell lymphoma (ALCL). They were instructed to perform highly sensitive ALK IHC for lung AdCA according to their standard protocols and to report cores as immunoreactive or non-immunoreactive. Information was collected on clone utilized, among other analytic variables.

Results: 112 laboratories participated. Rates of immunoreactivity in the 4 expected-positive lung AdCAs ranged from 29-60%, while 93% reported positivity for the ALCL. Rates of non-immunoreactivity in the expected negatives ranged from 96-100%. For the 4 expected-positive lung AdCAs, there was striking, clone-dependent variation (see Table).

	% Positive by Clone		
Core ID	ALK1 (n=65)	5A4 (n=25)	D5F3 (n=19)
2	42	52	100
6	27	16	95
7	16	16	89
8	49	60	100

Conclusions: ALK IHC-positivity in *ALK*-rearranged lung AdCA is clone dependent. The majority (60%) of laboratories utilized the ALK1 clone, despite known insensitivity of this clone in this setting. Clone 5A4 performed much more poorly than in published literature, suggesting challenges in antibody optimization. D5F3 performed well. These results highlight the importance of antibody selection, optimization, and clinical validation for predictive marker IHC testing.

1951 Utilization of Pathologists' Assistants in the Diagnostic Arena: Working Differently in the Changing Health Care Environment

Melissa M Blessing, David Schembri Wismayer, Diva R Salomao, Adam J Wood, Sarah E Kerr, Monica L Kendall, Kenneth M Smith, Carrie Y Inwards, Gary L Keeney, Mariam P Alexander. Mayo Clinic, Rochester, MN.

Background: Institutional consultation (IC) is the review of outside pathology of patients referred to the second institution for clinical care. This quality assurance review is known to effectively detect clinically significant diagnostic errors (0.6% of cases). Our IC service (ICS) reviews ~ 12563 cases/year and is staffed by 1.5 faculty pathologist (FP) FTE and 1 senior pathology trainee (TR) FTE. TR participation enhances work flow and FP efficiency; however due to high case volume, TRs preview only 40% of the cases. To provide additional support to FP, we piloted a quality improvement (QI) project using pathologists' assistants (PAs) on the ICS, aiming to enhance FP efficiency and satisfaction.

Design: The 1 month pilot project involved adding 1 FTE PA in the ICS along with FP and TR. 2 PAs participated in 2-week sessions each. Pre & post-pilot FP work-flow perception surveys (PS) were conducted. The components of the PS are shown in the table. Metrics of the QI project were hourly case sign out volume & percentage of cases previewed for FP.

Results: Pre and post-pilot PS participation was 55% & 77% respectively. Results are shown.

TABLE 1A: Areas where a Pathologists' Assistant could benefit ICS case preview structure.

	Pre-pilot (%)	Post-pilot(%)	P-value for chi-square test
ORGANIZATION	48	89	<0.001*
CASE REVIEW	38	33	0.459
CASE SHARING	29	45	0.094
SELECTION FOR TRANSFER	48	22	<0.001*
TRANSCRIPTION	29	33	0.540
CANCER TEMPLATE COMPLETION	43	60	0.016*

TABLE 1B: Perceived benefits of utilizing a Pathologists' Assistants in the ICS

	Pre-pilot (%)	Post-pilot(%)	P-value for chi-square test
MORE CASES REVIEWED	67	82	0.015*
MORE TIME SPENT/CASE	5	19	0.002*
MORE TRAINEE TEACHING	57	41	0.023*
PERSONAL CONSULTS	71	67	0.540
ADMINISTRATION	14	7	0.106
RESEARCH WORK	33	4	<0.001*

An increased positive perception (PP) was noted in 6 areas, after the pilot. In relation to ICS case preview, an increased PP was seen with: case organization, cancer template completion, transcription, and case sharing, with significant increase for case organization (p=<0.001) & cancer template completion (p=0.016). An increased PP to "benefits of ICS restructure" included time spent reviewing cases (p=0.002) & more cases reviewed (p=0.015). With the addition of a PA, 23% more cases were previewed (p=0.0001), equating to 0.5 FP FTE. There was an increase in volume of cases signed out/hour with turnaround time/case improving from a median of 8hr to 6hr 45 min. PAs flagged 22% of the major diagnostic discrepancies during the pilot period.

Conclusions: With the changing health care environment, declining reimbursement & pathologists' burn out, we are challenged to use available resources differently to meet increasing workloads. This QI project demonstrates that PAs can be utilized for ICS. Their participation enhances workplace productivity and efficiency.

1952 A Comparative Study of Colon and Rectal Tumor Specimens' Gross Examination by Biomedical Scientists, Residents and Pathologists: A Review of 688 Cases

Ana C Braga, Rita SS Oliveira, Marco Ferreira, Ana Lopes, Katia Reis, Leontino Lampreia, Manuela Novo, Rui Vaz, Rute Matos, Sara Marques. Hospital Prof. Doutor Fernando Fonseca, E.P.E., Amadora, Portugal.

Background: Nowadays, gross examination (GE) of surgical specimens in Portugal is performed by biomedical scientists (BMS), residents and pathologists. Our aim is to compare the GE of colon and rectal tumor specimens performed by these three professional groups.

Design: The GE of 688 specimens (531 BMS; 59 residents; 98 pathologists) was compared according to: number of blocks submitted, number of lymph nodes (LN) harvested, smallest LN and smallest metastatic LN. Statistical analysis was performed (Mann-Whitney U test, Kruskall-Wallis test and Chi-Square test; α=0,05).

Results: Statistical significance was observed between BMS, residents and pathologists in the number of blocks without LN (p<0,001), number of LN harvested (p<0,001), in the size of the smallest LN (p=0,003) and in the number of tumor blocks (p<0,001). No statistical significance was observed between BMS, residents and pathologists in the size of the smallest metastatic LN (p=0,625).

	Parameters (average)				
	Number of blocks without LN	Number of LN	Smallest LN (mm)	Smallest metastatic LN (mm)	Number of tumor blocks
BMS	15,42	23,89	1,672	4,765	7,09
Residents	16,17	22,66	1,754	4,273	8,93
Pathologists	10,32	13,68	2,026	4,167	5,90

Conclusions: In colon and rectal tumor specimens, BMS and residents show a better LN retrieval and BMS harvest the smallest LN. However, BMS and residents submit more blocks without LN compared to pathologists. These results suggest a promising BMS performance in the GE of the studied specimens.

1953 Cost Comparison of First Line Multiplex PCR Versus Screening with Influenza Single PCR for the Detection of Upper Respiratory Tract Viral Infections

John Cannatella, Benjamin Buelow, Lynn Pulliam. University of California, San Francisco, San Francisco, CA.

Background: PCR based diagnostics are a cornerstone of the microbiology lab. In upper respiratory infections (URI), viral PCR results affect choice of therapy, length of stay in the emergency department (ED) and the decision to admit. Recently, multiplex viral PCR assays have been shown to decrease length of stay and improve ED workflow. However, multiplex assays are more costly than single PCRs and may provide little additional actionable data. This study compares first line use of the Biofire FilmArray (BFA) versus Influenza (IVA/B) single PCR followed by second line BFA in IVA/B negative URI patients.

Design: All respiratory virus BFAs run at our institution between August 2014 and July 2015 were collected; 510 results were identified. Patient location (ED, in/out-patient), virus positivity, and virus type were recorded. Data analysis was performed in Microsoft Excel.
Results: Of 510 patient samples, 188 were positive for a viral pathogen (36.9%). The most common viruses were rhino/enterovirus (RV/EV; not separable on this assay), IVA/B, and respiratory syncytial virus (RSV; Figure 1). At their peak, IVA/B represented 25.2% of total samples (55% of total positives; January). The cost comparison of first line BFA versus IVA/B single PCR followed by second line BFA in IVA/B negative cases is shown in Table 1.
Figure 1: Frequency of Viruses Detected by BFA

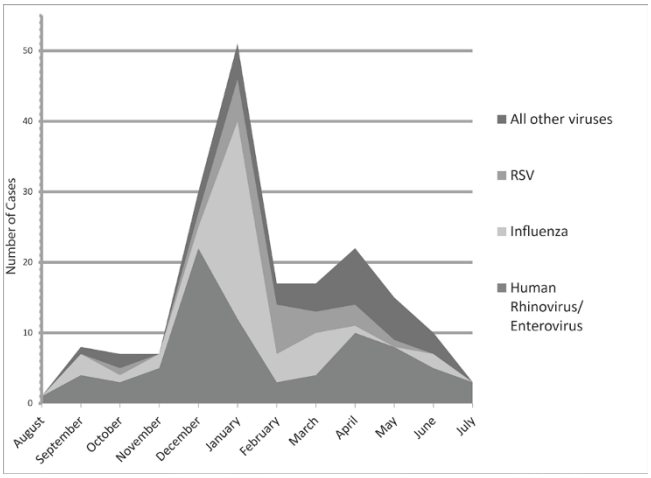


Table 1: Cost Comparison of First Versus Second Line BFA

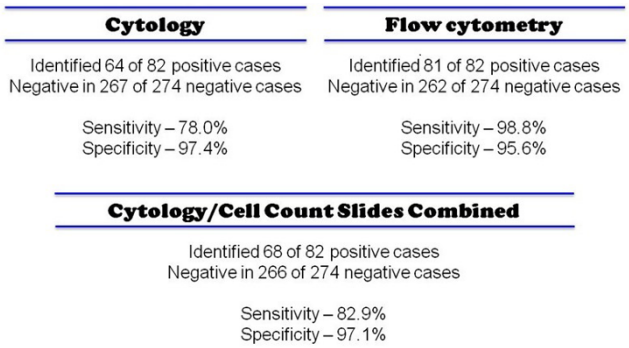
		Cost/Test ($)	# Tests	Total Cost ($)
12 Months	BFA All Samples	$129.00	510	$65,790.00
	IVA/B PCR	$37.10	510	$18,921.00
	BFA 2nd Line	$129.00	460	$59,340.00
				$78,261.00
Peak IVA/B Season	BFA All Samples	$129.00	111	$14,319.00
	IVA/B PCR	$37.10	111	$4,118.10
	BFA 2nd Line	$129.00	83	$10,707.00
				$14,825.10

Conclusions: A viral etiology was common for URI symptoms (36.9% of tested patients, Figure 1). First line use of the BFA was more cost effective than screening via IVA/B single PCR; this relationship inverts if IVA/B positives represent ≥29% of total samples, or if ≤71% of total samples undergo BFA (such as restricting BFA only to admitted IVA/B negative patients, as at our institution). In any case, even limited use of the BFA provides epidemiological data with potential utility both for research and infection control purposes.

1954 Cerebrospinal Fluid Specimen Evaluation in Patients with Hematopoietic Neoplasms: The Need for an Integrated Approach

Darren Buonocore, Mikhail Roshal, Samuel I McCash, Oscar Lin. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Cerebrospinal Fluid (CSF) evaluation plays an increasing role in the diagnosis and staging of patients with hematopoietic neoplasms. The evaluation is based on the morphological evaluation of the specimen and flow cytometry studies (FC). FC is known to be more sensitive than cytology, but it can identify small clonal proliferations of unknown clinical significance. The impact of the increased sensitivity of FC and its correlation with the morphological analysis of CSF needs to be evaluated to better guide clinical management.
Design: We retrospectively identified 356 CSF specimens sent for FC, cytology and cell count between March 2014 and December 2014, of which 79 from 45 different patients had discordant results. Seventy-four specimens had cytology specimens (ThinPrep, pap stained slides), FC specimens and slides used for cell count (Cytospin, air dried Giemsa stained slides) available for review. Each preparation was interpreted independent of the FC results as negative or abnormal. An extensive review of each patient's medical record was performed.
Results: The sensitivity of each of the specimens are described in Figure 1.



Alcohol fixed ThinPrep slides had the lowest sensitivity. Review of the Giemsa stained slides used for cell count increased the sensitivity of the morphological evaluation to 82.9%. Six patients who did not receive treatment based on initial negative morphological results were later found to have CSF involvement. Inability to initially corroborate the FC diagnosis resulted in four patients not being treated and two undergoing surgery for definitive diagnosis of diffuse large B-cell lymphoma. Incorporation of the Giemsa stained slides would have provided corroboration in three of the six cases.
Conclusions: ThinPrep slides had the lowest sensitivity, probably due to its larger screening areas combined with alcohol fixation. Accurate diagnosis of CSF specimens from patients with hematopoietic disease require integration of morphological findings from both alcohol fixed, Giemsa stained slides and flow cytometry results.

1955 Quality of Reporting in Predictive Biomarker Studies in Anatomic Pathology Journals

Justin Caron, Michael B Cohen, Robert L Schmidt. University of Utah School of Medicine, Salt Lake City, UT.

Background: Studies on predictive biomarkers are frequently published in pathology journals. Such prognostic studies are complex, and guidelines have been published that outline the data that should be reported by each study to enable readers to evaluate the quality of the research. Complete reporting is also critical for the production of high-quality systematic reviews and meta-analyses. Reporting guidelines have not been widely adopted by pathology journals. Journals in other disciplines often require authors to submit checklists to ensure adherence to reporting guidelines; however, this is uncommon in pathology. This study evaluated the quality of reporting of prognostic studies published in pathology journals.
Design: We identified all prognostic studies published in the *American Journal of Clinical Pathology*, the *American Journal of Surgical Pathology*, and *Modern Pathology* published between January and August 2015. We randomly selected ten studies from each journal. We evaluated each study using the REMARK reporting guidelines for prognostic studies. A scoring statistic was created based on the elements in the REMARK guideline. Adherence was expressed as percent items reported. REMARK items with multiple components were scored by component and averaged to obtain an item score. Each study was independently scored by two referees. Disagreements were resolved by discussion for items that had less than 80% concordance.
Results: The initial inter-rater agreement was 71%. Agreement increased to 95% following discussion and rescoring of items with low (i.e., less than 80%) agreement. On average, authors reported 51% of the items listed in the REMARK guideline. There was no significant difference between journals. The following items were poorly reported: power analysis (reporting frequency = 3%), flow diagrams (4%) or REMARK profiles (0%), correlations between predictors (3%), or model validation or assumption checking (2%). No study described methods for handling missing values or reported results of missing value analysis. Only one study divided data into a training set and validation set. None of the studies included all of the required information.
Conclusions: Authors of prognostic studies fail to report the items recommended by the REMARK guideline. The absence of this information significantly limits the value of these studies as evidence for inclusion in systematic reviews, meta-analyses, cost-effectiveness analysis, and practice guidelines.

1956 Pathology Review of Research Biopsies Highlights Inter- and Intra-Tumor Variability: Towards Improving Quality and Utility for Next-Generation Biospecimen Research

Dianne Chadwick, Michael H Roehrl. University Health Network, Toronto, ON, Canada; Memorial Sloan Kettering Cancer Center, New York, NY.

Background: High quality research biospecimens are key elements of success towards developing truly personalized medicine of the future. Research biopsies are being increasingly used to obtain tissue from patients with cancer, either when no surgical resection has been performed, or to monitor response to therapy. We report on the histological quality of research biopsies banked with our program towards determining their suitability for subsequent molecular analysis.
Design: Research biopsies were obtained under institutionally approved research protocols from consented patients with either localized prostate cancer (pre and post radiotherapy), advanced melanoma, or with pancreatic cancer liver metastases. Up to 12 cores were obtained per patient. Cores were immediately snap frozen in OCT, fixed, and embedded in paraffin, or transferred fresh into culture medium for xenografting into NOD SKID mice. Reviews were performed on H&E slides from fixed and frozen tissue blocks.

Results: H&Es of 99 cores from 33 patients were reviewed. Most of the cores (59/99) were positive for malignancy: 34/65 prostate cancer, 21/26 melanoma and 4/8 pancreatic cancer liver metastases cores contained tumor, ranging from 90% to less than 5% of represented tissue. Although patient-derived murine xenografts were successfully established in all 5 cases of pancreatic cancer liver metastases from fresh cores taken at the same time, only 1/5 cases (and 1/8 cores) had sufficient tumor cellularity for DNA and RNA extraction for whole genome and RNA sequencing. Research biopsies from 8 patients were completely negative for malignancy: 15 cores from 3 patients with prostate cancer, 5 cores from 3 patients with advanced melanoma localized to the arm, lymph node and lung, and all 3 cores from 2 cases of pancreatic cancer liver metastases.
Conclusions: Pathology review of research biopsies banked with our program has demonstrated variability in quality between patients with diverse and similar cancer types, as well as between cores from the same patient. The results highlight the importance of assessing tumor cellularity and histological quality prior to downstream analysis. Through close monitoring of preanalytical variables such as disease type, tissue site, and patient pretreatment and tracking downstream results such as histological quality, integrity of molecular analytes such as DNA and RNA, and maintenance of cell viability through patient-derived xenografts, it may be possible to identify ways to optimize the utility of these important next generation biospecimens.

1957 Evaluating Pathologist Accuracy in Estimation of Percentage of Malignant Cells

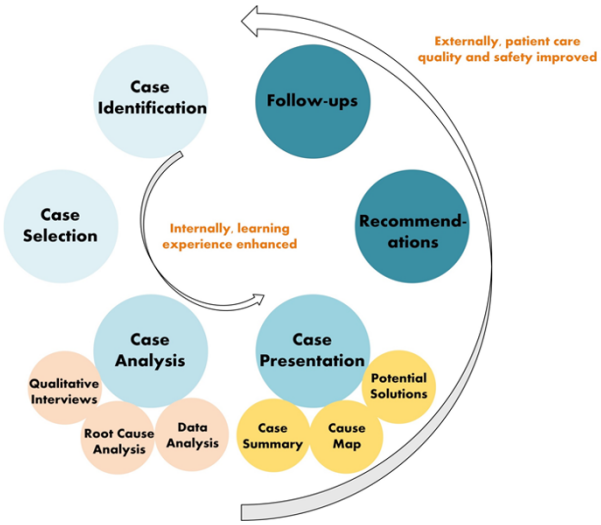
Hui Chen, Russell Broaddus, Bedia A Barkoh, Ronald Abraham, Rajesh Singh, Raja Luthra, Sinchita Roy Chowdhuri. MD Anderson, Houston, TX.

Background: Accurate estimation of invasive tumor fraction in solid tumors submitted for molecular testing is a critical variable for testing success. The interpretation of comprehensive and advanced diagnostic genomic tests such as next-generation sequencing (NGS) and SNP microarray requires the precise estimate of tumor fraction to avoid false-negative test results. Prior studies based on the College of American Pathologists Survey have shown low inter-laboratory precision among pathologists using visual estimation for determining tumor fraction. To minimize variability in tumor fraction estimation, our division has initiated a quality improvement project since 2013 by designating a tissue qualification laboratory (TQL) staffed with dedicated pathologists with experience in molecular diagnostics to select tissue sections and to evaluate tumor fraction for molecular testing. , We retrospectively reviewed core needle biopsy (CNB) samples to determine visual estimation accuracy among TQL pathologists.
Design: We evaluated 121 consecutive malignant CNBs performed by interventional radiologists, 2013-2014. Tumor enrichment was performed by selecting and circling areas within the tissue section by TQL pathologist 1. Each case was evaluated independently by at least 1 other TQL pathologist and a subset of cases were reviewed by a third TQL pathologist. The discordant slides were reviewed together at the end of the study. NGS was performed on 22 cases using the Ampliseq Cancer Hotspot v2 panel on the Ion Torrent PGM (Life Technologies, CA). Variant frequency was recorded and normalized with the tumor fraction estimate.
Results: The tumor fractions ranged from <10% to 90% (median 40%, mean 41%). The tumor fraction estimate between TQL pathologists 1 and 2 showed excellent concordance with identical results in 59 cases (49%) and <10% difference in 61 cases (50%), with a single case (<1%) showing >10% difference. Variant frequency obtained from NGS analysis is an alternative, though imprecise, method for estimation of tumor fraction. Using the variant frequency as a rough estimate of the tumor content of the specimen, the averaged tumor fraction estimates of the 3 TQL pathologists were within 10% of the adjusted variant frequency in 13 cases (59%) and between 11-15% in 8 cases (36%), with a single case at 23% (4%).
Conclusions: Our findings suggests that visual estimation of tumor fraction by TQL pathologists is fairly accurate and that significant estimation errors can be avoided when using dedicated and experienced pathologists for molecular qualification of solid tumors.

1958 Pathology M&M Rounds: A Focus on Transparency and Process Improvement

Yigu Chen, Jeffrey Goldsmith, Yael K Heher. Beth Israel Deaconess Medical Center, Boston, MA.

Background: Morbidity and Mortality (M&M) Rounds are typically reserved for clinical review of adverse events. Pathologists frequently participate in extradepartmental M&Ms (Surgery and Medicine) but rarely hold our own. We implemented pathology-specific M&M Rounds for review of adverse events and near-misses. By promoting transparency and incident review previously held behind closed doors, we were able to share root cause analyses and promote process improvement.
Design: Monthly M&M conferences were established using a standardized model that included case identification, analysis, and process improvement actions. We utilized quality improvement tools such as process mapping, cause mapping, and fishbone analysis to investigate potential systems vulnerabilities. After implementing the recommended interventions, we held follow-up sessions to provide feedback and discuss continuous improvement opportunities. Extradepartmental providers were included in all levels of the M&M process.



Results: Monthly M&M conferences have been successfully held since 2012. 31 conferences have addressed adverse events from the operating room (6), grossing room (4), histology laboratory (3), pathology signout (5), clinical pathology (7), and other sites of care (6). Numerous vulnerabilities in our processes have been improved, such as standardization of intraoperative consultations, minimization of labeling error, and optimization of tissue handoffs. Faculty, trainees, and frontline workers have been empowered at every step. The M&M model equips participants with necessary quality management tools while growing a culture of transparency and continuous improvement.
Conclusions: Pathology-specific M&M conferences transform adverse or near-miss events from sources of frustration and anxiety to sources of learning and improvement. They facilitate discussion and innovation by including and empowering all levels of Pathology staff and stressing a culture of continuous improvement. M&M rounds at BIDMC, which are unique at a national level, have become a core part of our "Just Culture" and have served as an effective mechanism to develop and ameliorate quality and safety performance overall.

1959 Adopting the Quality Dashboard in Pathology: Real-Time Data Monitoring and Improvement

Yigu Chen, Gina McCormack, Yael K Heher. Beth Israel Deaconess Medical Center, Boston, MA.

Background: The dashboard format of quality metric monitoring has been widely accepted as the industry standard for real-time data representation and monitoring. Although laboratories track numerous quality metrics, indicator data are often collected and reviewed using outdated and manual techniques. Standardized quality data reporting templates do not exist, making data difficult to interpret and timely improvement efforts challenging. We chose to transform our process, adopting a real-time digital Quality Dashboard (QD).
Design: A multidisciplinary team was assembled composed of the Quality Medical Director, a Process Improvement Specialist, the Laboratory Operations Director, and Safety and Compliance officers (3). Team goals included a full review and update of quality metrics with a focus on patient care and clinician experience, as well as subsequent monitoring of established targets for improvement efforts. A standardized data collection template was designed and utilized by lab managers for uniform input of electronic performance data. Using formulas embedded into the template design, graphics such as run charts, control charts, and percentage charts were automatically generated and inserted into the QD. A simple visual red/green color-coded trigger system, based on the Toyota Production System "andon", was used to monitor real-time performance and cue targeted improvement efforts.
Results: The QD format was rolled out in March 2015 and has been reviewed by lab managers and medical directors on a bi-monthly basis. 11 of 14 lab divisions have modified their quality and performance indicators based on the QD, shifting their focus from quality targets that had already been achieved to those in need of improvement. Red/green "andon" triggers have allowed rapid visual performance feedback and have given rise to 12 quality improvement projects in anatomic pathology (5), hematology (2), clinical chemistry (1), blood bank (2), autopsy (1) and microbiology (1). Statistical control charts have determined process reliability and highlighted irregular workflows in need of redesign. Since the QD rollout, quality data review has been transformed from a cursory and frustrating process to a timely and proactive culture of continuous improvement.
Conclusions: Outdated narrative and manual data collection, analysis, and presentation methods impede the effectiveness and timeliness of quality management. Creation of a comprehensive digital QD enabled us to monitor overall laboratory performance in real-time, and served as a guide for quality and process improvement activities.

1960 Quality Assurance Aspects of implementing BRAFV600E Immunohistochemistry (IHC) Assay in Universal Lynch Syndrome Screening (ULS) for Colorectal Cancer (CRC): A Single Institution Experience

Zongming E Chen, Angela Bitting, Nefize Kip, Fan Lin. Geisinger, Danville, PA.

Background: Reflex BRAF mutation analysis is a necessary triaging step in the screening algorithm of ULS for CRC. Studies have shown BRAF V600E can be detected by IHC with sensitivity and specificity comparable to DNA-based methods. In clinical settings, the IHC assay may help to improve turn-around time and reduce cost. How to properly implement the assay clinically is still controversial among labs. We decide to share our on-going experience regarding quality assurance in the process.

Design: VE1 antibody was used. PCR was the DNA-based method. Three phases were planned for the implementation process, each emphasizing unique quality assurance aspects. Phase 1 focused on staining optimization and validation. Phase 2 focused on pathologist training, standardization of reporting and prospective clinical validation. Phase 3 will focus on periodic assay performance check and pathologists' proficiency review.

Results: Phase 1 was completed in two steps. Initial validation was performed using tissue microarrays (TMA). 152 cores of CRC were tested. 26 (17%) cores were positive, 8 (5%) equivocal and the remaining negative. 86 cores of normal colon were also tested negative. Additional validation was performed using whole tissue sections. 20 CRCs with known BRAFV600E mutation status were tested blindly and a perfect concordance was achieved. Phase 2 started with training pathologists on proper stain interpretation. To reduce inter-observer variability, only 4 pathologists were charged for the task of reporting BRAFV600E stains. To standardize reporting format, an electronic template with three defined categories (positive, equivocal and negative) as selecting functions was used. In this phase, 11 CRCs with MLH1/PMS2 loss were tested prospectively using both IHC and PCR methods simultaneously. IHC results were interpreted as 8 positive and 1 negative, completely in agreement with the results from DNA-based method. 2 cases were deemed as equivocal by IHC; 1 tested positive and 1 negative by PCR. The negative case was further confirmed by absences of MLH1 promoter hypermethylation. The results helped to decide that all IHC equivocal or negative cases to be confirmed by PCR method in phase 3 testing which is to be completed in a year.

Conclusions: Quality assurance is the key to implement BRAFV600E IHC as a useful clinical assay in ULS for CRC. The experience gained has general application for implementing other IHC predicative biomarkers in the future.

1961 Image Documented Surgical Pathology (ID-SP) to Enhance Patient Safety - Lean Redesign of the Value Stream Steps from Gross Examination to Signout

Dhananjay A Chitale, Jason Wozniak, Nelson Main, Ruan Varney, Mark Tuthill, Richard J Zarbo. Henry Ford Hospital, Detroit, MI.

Background: Mis-identification (Mis-ID) and Mis-communication (Mis-COM) defects are frequent causes of potentially significant medical errors that threaten patient safety. The hand-off nature of surgical pathology processes from specimen collection to signout affords opportunities for Mis-ID/Mis-COM failures. To further enhance safer surgical pathology, we have explored process and technologic innovations that leverage the maxim "a picture is worth a thousand words" by integrating ID-SP digital workstation employing images attached in the lab information system that visually document all required information at gross examination to be used by downstream workstations.

Design: Times to perform all steps of grossing and documentation were assessed comparing 25 biopsies grossed by a single pathology assistant with our traditional grossing protocol (TGP), dictationless & wordless, to ID-SP protocol developed for the LeanSTATION Bx digital macro-imaging system (Milestone Medical, Kalamazoo, MI). ID-SP documentation gross protocol consisted of sequential digital image recordings: 1-requisition, 2- specimen container label as received and barcoded cassette, 3-container contents as received, 4- tissue placed in cassette with superimposed 1 mm electronic measuring grid.

Results: Average time required for ID-SP gross was 76 seconds per specimen vs. 37 seconds for TGP. Histology personnel could refer to gross specimen descriptions at time of embedding whereas actual images of submitted tissues in cassettes were available attached to the case in Pathology PACS system (Apollo PACS, Inc, Falls Church, VA). These images were also available to downstream histology personnel at cutting stations. Pathologists at the time of signout had additional visual quality control checks to confirm patient identification of requisitions, container and cassette labels and submitted tissue.

Conclusions: Grossing time of ID-SP protocol was double TGP, but the image protocol provided additional assurance of catching Mis_ID and Mis-COM errors with prospective visual quality control tracking of requisitions and specimens at each workstation from gross to pathologist. In our experience the time difference expended is more than recouped in the average rework time of 8 man-hours wasted in resolving a MIS-ID case searching for empty specimen containers, interviewing clinical & pathology personnel, performing DNA profiling and amending reports. Further efficiencies in some practices may be obtained with ID-SP work design by eliminating time & bottlenecks in transcription and report correction

1962 Next Generation Sequencing for Epilepsy, Mitochondrial Disease, and Developmental Delay: One Pediatric Hospital's Experience

Jessica L Davis, Bonnie Cole, Jessie Contra, Jane Dickerson. Seattle Children's Hospital, Seattle, WA.

Background: Next generation sequencing (NGS) is a promising tool for evaluation of rare pediatric genetic diseases, especially those causing developmental delay (DD), epilepsy, and mitochondrial dysfunction. Identification of mutations in certain genes has the potential to influence medication or dietary alterations, or may change the ordering practice of radiology studies or referrals to other specialists. Many such gene panels are relatively new, and therefore, their clinical usefulness remains unknown. We sought to evaluate the spectrum of results obtained through this type of NGS, as well as, the clinical utility of such testing in our patient population.

Design: Most of these panels at our hospital are sent to one reference lab. All NGS epilepsy, mitochondrial, and DD panels sent to this reference laboratory between July 2013-2015 were included in this study. Results were reviewed, including recording number of variants and significance of each variant identified, correlated with an in-depth chart review, including ordering provider information, clinical phenotype, and management pre- and post results. Cost analysis was also performed.

Results: Twenty-two NGS tests were sent out to one reference lab over the two year study period, ordered exclusively by neurologists and geneticists. Variants of unknown significance (VUS) were detected in 17/22 patients, with an average of 3.2 VUS generated per test. Only one likely pathogenic mutation was identified. This single pathogenic mutation was a heterozygous *KCNQ2* mutation in a patient with epilepsy. The chart review did not reveal any documented change in clinical management of this patient. Total cost of testing to our hospital for NGS testing sent to this one reference laboratory was $144,215, with an average cost per patient of $5,341. These tests took on average 62.5 days to be resulted (range 15-145 days).

Conclusions: In our patient population, NGS panels for DD, epilepsy, and mitochondrial dysfunction sent to one major reference laboratory had a positive hit rate of less then 5% with a cost-per-positive test of $144,215. So far, none of these genetic test results have led to a documented change in clinical care. Providers ordering these tests should be aware of the low likelihood of finding pathogenic mutations and should take this information into account when considering ordering these tests, particularly given that many patients may be financially liable for these expensive laboratory tests.

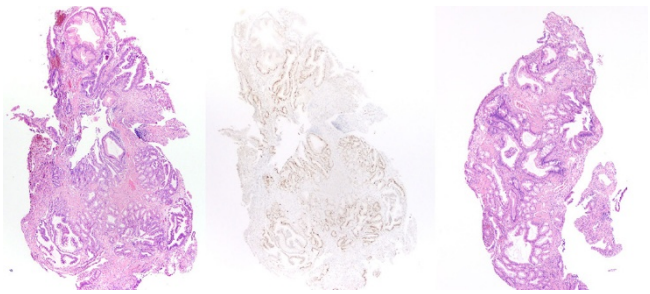
1963 Duodenal Polyps Resembling Peptic Duodenitis in Patients with Hereditary Cancer Syndromes

Kyle Devins, Lisa Wang, Maryam Zenali. SUNY Upstate Medical University, Syracuse, NY; University of Vermont, Burlington, VT.

Background: An increased tendency in developing tubular adenomas, hyperplastic, and mixed polyps, has been documented in patients with Lynch Syndrome (LS) and attenuated adenomatous polyposis, amongst others. We report histologic features closely resembling peptic duodenitis in polyps from patients with known LS (4/10) or family history of cancer (3/10).

Design: After diagnosing an index case, study cohort included 10 duodenal biopsies from 4 patients with known LS. In addition, 4 duodenal biopsies and 2 resections with similar histologic features were collected from 6 patients without confirmed LS. Characteristics of patients and specimens are summarized in figure 2. Genetic testing for mismatch repair (MMR) proteins was performed in 4/16 specimens upon feasibility.

Results: Polyps had foci with foveolar type epithelium overlying proliferative Brunner's glands, closely resembling peptic duodenitis. There were also scattered hyperplastic/dilated crypts. See H&E images. MMR staining showed total loss of MSH6 expression in 1, while heterogeneous MSH6 expression in 2 others. Another case had heterogeneous expression of both MSH6 and PMS2. See IHC image with heterogeneous staining.



Age	Gender	Clinical History	Specimen	Diagnoses Including Original Descriptions	Dysplasia
46	M	Lynch Syndrome	Duodenal biopsy	Foveolar adenoma	None
			Duodenal biopsy	Foveolar adenoma	None
84	M	Recurrent duodenal polyps	Duodenal biopsy	Foveolar and Brunner's gland hyperplasia	None
75	F	Lynch Syndrome	Duodenal biopsy	Peptic duodenitis with hamartomatous features, heterogeneous MSH6 expression	None
			Duodenal biopsy	Polypoid duodenal mucosa	None
			Duodenal biopsy	Peptic duodenitis with hamartomatous features	None
64	M	Lynch syndrome	Duodenal biopsy	Active duodenitis	Indefinite
			Duodenal biopsy	Active duodenitis with foveolar metaplasia	None
			Duodenal biopsies	Foveolar adenoma and hamartomatous polyps	None
			Duodenal biopsy	Hamartomatous/hyperplastic mucosa, heterogeneous MSH6 expression	Low grade
62	M	Lynch syndrome (Muir-Torre)	Duodenal biopsy	Peptic duodenitis	None
64	F	Family history of cancer in 1st degree relatives	Duodenal biopsy	Adenoma with low grade dysplasia	Low grade
35	F	Unknown family history	Segmental duodenectomy	Foveolar adenoma, Brunner's gland hyperplasia, heterogeneous MSH6 and PMS2 expression	Low grade
54	M	Unknown family history	Duodenal biopsy	Peptic duodenitis with hamartomatous features	None
54	F	Family history of cancer in 1st degree relatives	Segmental duodenectomy	Adenoma with focal osseous metaplasia, loss of MSH6 expression	Focal high grade
85	M	Family history of cancer in 1st degree relatives	Duodenal biopsy	Foveolar adenoma with hamartomatous features	None

Conclusions: Hamartomatous polyps in hereditary syndromes such as LS can closely resemble peptic duodenitis on limited biopsy and lead to misdiagnosis. To the authors' knowledge, this finding is not reported in the literature. Attention to subtle features, such as irregular/cystic crypts with hyperplastic change and haphazard appearance, is

critical to avoid misinterpretation. In an incidental specimen categorized otherwise as "polypoid peptic duodenitis," the aforementioned features should prompt further work up including MMR testing and/or detailed clinical history to exclude the possibility of a hereditary disease.

1964 Fine Needle Aspirations of the Lung: Impact of Interobserver Variability amongst Cytopathologists Can Affect the Diagnosis

Zachary J Dureau, Brian Robinson, Momin T Siddiqui, Uma Krishnamurti, Marina Mosunjac, Talaat S Tadros, Wei Wei Shi, Vaidehi Avadhani, Alessandra Schmitt, George Birdsong, Gabriela M Oprea-Ilies. Emory University School of Medicine, Atlanta, GA.

Background: Interpreting malignant pulmonary fine needle aspirations (FNA) may be challenging, especially with scant cellularity; ancillary testing is often necessary to subclassify these neoplasms. Treatment and prognosis rest upon an accurate diagnosis made by cytopathologists. There are few studies in the literature quantifying the degree of diagnostic variability amongst pathologists in a setting without immunohistochemistry (IHC).

Design: FNAs between 2014-15 in patients with a primary or metastatic lung carcinomas with diagnoses confirmed via IHC or departmental consensus were selected [37 adenocarcinomas (ADC) or non-small cell carcinoma favor adenocarcinoma (NSCLC/ADC), 31 squamous cell carcinomas (SqCC), 3 carcinoid tumors (CT), 15 small cell carcinomas (SMC), and 3 non-small cell carcinoma not otherwise specified (NSCLC/NOS)]. Seven pathologists with an average of 12 yrs experience (range: 1-21 yrs) rendered a diagnosis for each case without knowledge of final diagnosis. They blindly interpreted three preparations from each case: a Romanowsky variant stain (DQ), a Papanicolaou stain (PAP), and a hematoxylin and eosin stained cell block (HE).

Results: Thirty-one cases were identified. Eighty-nine total preparations, consisting of 29 DQs, 30 PAPs, and 30 HEs, were interpreted. Overall concordance was 63% (range: 48-69%). The kappa value for all preparations was 0.40. Kappa values for DQ, PAP, and HE preparations were 0.31, 0.38, and 0.50, respectively.

Conclusions: 1. Interobserver agreement was only fair overall when calculated for DQ and PAP stains, and moderate for the HE stained cell block.

2. Interobserver agreement was strongest for a diagnosis of SMC.

3. Interobserver agreement was weakest for a diagnosis of NSCLC/NOS.

4. As expected, diagnostic concordance generally increases with more experience.

5. Our study confirmed that cytomorphology alone is not enough to diagnose these tumors. Use of IHC markers is helpful in classifying poorly differentiated carcinomas to guide patient management. An IHC panel delineating ADC, SqCC, and when indicated by morphology, SMC, would decrease variability between pathologists, and ensure appropriate patient treatment. Turnaround time may be reduced if the IHC panel can be ordered up front.

6. It is important to obtain sufficient material at the time of FNA for ancillary testing including molecular genetics. Dedicated passes are often beneficial for this purpose.

1965 The Role of p16 in the Classification of Anal Squamous Intraepithelial Lesions: Evaluation of Interobserver Variability

Zachary J Dureau, Uma Krishnamurti, Mario Mosunjac, Talaat S Tadros, Marina Mosunjac. Emory University School of Medicine, Atlanta, GA.

Background: Morphologic interpretation of anal squamous intraepithelial lesions is a diagnostic dilemma for many pathologists. Block positivity with p16 immunostaining can be used to determine the presence of a high grade squamous intraepithelial lesion (HSIL), and its use has been accepted as a standard of care with cervical lesions. There are limited studies in the literature investigating the utility of p16 in accurately distinguishing between HSIL and low grade anal squamous intraepithelial lesions (LSIL), and assessing the degree of observer variability seen when p16 is not available.

Design: Fifty cases of anal biopsies from 2014 were extracted from the pathology database. Twenty-two (44%) of these biopsies did not utilize p16 to render a diagnosis, while 28 (56%) did [in accordance with the Lower Anogenital Standard Terminology (LAST) recommendation]. Four pathologists with an average of 15 years of experience blindly evaluated the hematoxylin and eosin (HE) preparations only from these cases and not the p16, and rendered a diagnosis of LSIL, encompassing AIN I, or HSIL, encompassing AIN II and AIN III. Breakdown of cases evaluated is as follows: 18 AIN I (LSIL), 19 AIN II (HSIL), and 13 AIN III (HSIL). Statistical analysis was used to compare the accuracy of diagnoses with and without use of p16 as a marker of HSIL.

Results: Overall diagnostic accuracy was 69%. Accuracy for cases where p16 was not required for diagnosis was 82%. In cases where p16 was utilized but unavailable to the reviewers accuracy dropped to 58%. In cases where p16 was utilized, the result of the immunostain upgraded the observers' responses from LSIL to HSIL in 25% of observers' number of responses (28/112) or 11/20 cases, and downgraded from HSIL to LSIL in 17% of observers' overall responses (19/112), or 6/8 cases. The kappa (K) value in cases without p16 i.e where p16 was not required to make a diagnosis is 0.68. K values overall and for cases where p16 was used but unavailable to the reviewers are 0.58 and 0.49, respectively.

Conclusions: In our study we found that the use of p16 greatly aided in diagnostic concordance. Interobservor agreement was substantial when there is no need for p16; however, when its use is indicated and it is unavailable, agreement is moderate at best. This study reiterates the importance of utilizing p16 as recommended by the LAST guidelines, particularly when evaluating difficult to interpret anal biopsies. This will ensure diagnostic accuracy and the best possible patient care.

1966 Initial Validation of Whole Slide Imaging (WSI) for Use in Frozen Section Consultation at Stanford University

Sebastian Fernandez-Pol, Eugene Carneal, Mathew Rumery, Peyman Samghabadi, Brent Tan. Stanford University, Stanford, CA.

Background: Published studies have shown that WSI can be used to accurately diagnose frozen sections when the interpreting pathologist is located at a site remote from the surgery. The published rate of concordance between glass slides and virtual slides is high and similar to the rate of concordance when comparing glass to glass. To provide the option of remote secondary consultation during intraoperative frozen sections at the Stanford South Bay Cancer Center (located ~21 miles from the Stanford Department of Pathology), an Aperio Scanscope CS2 was acquired. This study was performed as an initial evaluation of intraobserver variability among pathologists at Stanford University, comparing glass slides to WSI from the Scanscope for intraoperative frozen sections.

Design: We searched our pathology database for cases with frozen sections performed in 2014 by one of 8 attending pathologists. Sixty-two cases were selected and 1 representative level of each case was scanned at 20x magnification. The 62 cases were shown to one of 8 attending pathologists, with each pathologist interpreting the same cases that they had originally interpreted at the time of the frozen section. At the time of interpretation, the pathologists had access to the clinical history as written in the original requisition sheet provided with the specimen. The pathologists received no prior formal training or practice before interpretation of the WSI. In addition, one fellow reviewed all of the cases to estimate the degree of interobserver variability.

Results: Of the 62 slides that were initially scanned, 7 cases were excluded for the following reasons: 2 were out of focus on the WSI, 2 cases had folded tissue that precluded interpretation, 3 cases were lost in the data archives prior to interpretation. Of the 55 cases that were interpreted, the diagnosis rendered using the virtual slide was concordant in 50 (91%) when compared with the original intraoperatively rendered diagnosis. Major discordance was seen in 3 cases (5%) and minor discordance was seen in 2 cases (4%). Kappa was estimated to be 0.79. The interobserver concordance was found to be 95% (kappa 0.89).

Conclusions: This preliminary validation show that the intraobserver variability for pathologists at our institution when using virtual slides is comparable to that seen by other authors. Our findings suggest that WSI may be an effect tool to provide remote secondary consultation support during intraoperative frozen sections.

1967 Fresh Cut Versus Stored Cut Paraffin-Embedded Tissue: Effect on Immunohistochemical Staining for Common Breast Cancer Markers

Catherine Forse, Dushanthi Pinnaduwage, Shelley Bull, Anna Marie Mulligan, Irene Andrulis. University of Toronto, Toronto, ON, Canada; Mount Sinai Hospital, Toronto, ON, Canada; University Health Network, Toronto, ON, Canada.

Background: The proper handling of unstained cut paraffin slides has been a matter of debate, with several studies demonstrating loss of antigenicity with prolonged storage at -20 °C and/or room temperature. In particular, stored slides have shown significant loss of Human epidermal growth factor receptor 2 (HER2) staining after storage at room temperature for 6 months. The purpose of this study was to determine if long term storage of unstained slides at -80 °C would impact the staining intensity and expression distribution of cytokeratin 5 (CK5), epidermal growth factor receptor (EGFR), Ki67, estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2).

Design: The staining pattern of invasive ductal breast carcinoma slides (n=34-64) stored at -80 °C for at least 10 years was compared to the staining pattern of fresh cut slides. The Allred scoring method was used to score ER (0,2=negative, 3-8=positive), CK5 (≥4=positive) and EGFR (≥4=positive). ASCO/CAP guidelines were used to assess HER2 (0/1+, 2+ or 3+). Ki67 positive tumor cells were determined based on the proportion of cells stained of any intensity, with 14% staining used as a cut-off. Agreement was assessed using concordance rates and chance corrected agreement statistics.

Results: The concordance between stored and fresh cut slides for ER, CK5, EGFR and HER2 was high with chance-corrected agreements of 0.94, 0.92, 0.87 and 0.86 respectively. The chance-corrected agreement for Ki67, however, was 0.51. For Ki67, nine tumors were called low on stored slides but high on fresh cut slides, and one tumor was called high on stored slides but low on fresh cut slides.

Conclusions: Long term storage of cut unstained slides at -80 °C does not significantly impact the scoring interpretation of ER, CK5, EGFR and HER2.

1968 Upfront Immunohistochemistry to Evaluate Sentinel Lymph Nodes in Malignant Melanoma: Burden or Benefit?

William Frampton, Dani S Zander, Dipti M Karamchandani. Hershey Medical Center, Hershey, PA.

Background: Immunohistochemistry (IHC) has been variably used, with hematoxylin-eosin staining (H&E), to facilitate detection of small melanoma deposits in lymph nodes. However, IHC increases costs and demands on laboratory resources. This study assessed the utility of upfront IHC as a component of the standard protocol for evaluation of sentinel lymph nodes (SLNs) from patients with malignant melanoma (MM).

Design: Multiple institutions in the USA and Canada were electronically surveyed to obtain information about their protocols for evaluation of SLNs excised for possible metastasis of MM. The pathology database at our medical center was searched for all SLN excisions performed on patients with MM from October, 2013 through June, 2015. All available H&E, S100, HMB-45, and Melan-A slides from these cases were re-examined. Metastases were classified as macrometastasis (>2.0 mm), micrometastasis (0.2-2.0 mm) or isolated tumor cells (ITCs, <0.2 mm).

Results: Fourteen institutions provided information about their standard protocols. Ten (including our institution) order IHC upfront (before H&E review) and four order IHC only if an H&E appears negative or suspicious for metastasis. 340 SLNs submitted in

494 tissue blocks from 150 consecutive patients (median age: 61 years; range 23-94 years) with MM were evaluated, revealing 21 (associated 37 blocks; 7.5%) that were positive on H&E (6.2%), showing macrometastasis (n=10), micrometastasis (n=9), and ITCs (n=2). S100, Melan-A, and/or HMB-45 identified an additional 9 (associated 14 blocks) positive SLNs (2.6%), which included micrometastases (n=3) and ITCs (n=6) that were not recognized on initial H&E review. The majority of SLNs (310, 91.2%) and associated blocks (443, 89.7%) were negative on H&E and with all three IHC stains.
Conclusions: Protocols for SLN examination in MM differ among health care centers, with the majority ordering IHC upfront. However, H&E stains alone enabled identification of all macrometastases and a majority of micrometastases; upfront IHC performed in these cases was likely not required. Although IHC staining increases detection of small metastatic MM deposits, upfront IHC generates a significant increase in costs and resource utilization. Instead of upfront ordering of IHC, it may be worthwhile to order IHC as a second step on negative or suspicious cases after H&E review, to improve detection of small metastatic deposits while reducing unnecessary costs.

1969 Communication in a Pathology Report: Differences between Pathology Attendings, Residents and Clinicians

Blake Gibson, Robert R Klein, Ronald S Weinstein, Erika Bracamonte. University of Arizona, Tucson, AZ.
Background: Pathology reports are the main modality in which pathology results are communicated to other physicians on the care team. Little data has been generated related to pathologist's intentions in communicating diagnostic certainty in the report vs. what is understood by its readers. This study compared perceived diagnostic certainty between pathology attendings, residents and clinicians through common diagnostic phrases and scenarios.
Design: Anonymous surveys were completed by 4 practicing pathology attendings, 10 pathology residents, 18 attending clinicians and 16 resident clinicians. All participants rated % certainty associated with 7 diagnostic terms: "diagnostic of", "consistent with", "we favor", "suggestive of", "suspicious for", "compatible with" and "we cannot rule out". Pathologists answered 2 qualitative questions related to utility of immunohistochemistry (IHC) and the diagnostic comment. Clinicians answered additional questions regarding utility of other communication methods (tumor boards/conferences, phone calls, email, text message) in clarifying diagnoses and how often the diagnostic comment is read.
Results: A wide range in % certainty was found for each of the 7 diagnostic phrases. "Diagnostic of" conveyed the best agreement as to % certainty, while % certainty for "compatible with" ranged from 10% to 90%. There was a lack of consensus between pathologists regarding the impact of unavailable or contradictory IHC results. While 75% of attending pathologists and 60% of pathology residents believe the diagnostic comment clarifies the diagnosis "very well", only 72% of attending and 50% of resident clinicians "always" read the comment section, and 28% of attending clinicians and 50% of resident clinicians "sometimes" read the comment.
Conclusions: The understood level of certainty for diagnostic phrases varies widely amongst pathology attendings, pathology residents and clinicians. This may impact pathology training as trainees navigate which phrases to use and in what context. Efforts to standardize use of diagnostic terms may improve communication. Clinicians regard tumor board/conferences, face to face meetings and phone calls as best ways to clarify a diagnosis. While pathologists often rely on the diagnostic comment to clarify uncertainty, a significant number of clinicians do not regularly read the comment.

1970 HPV Status and Other Contributing Factors to Negative Pap Tests in Women with Biopsy Proven High Grade Cervical Lesions

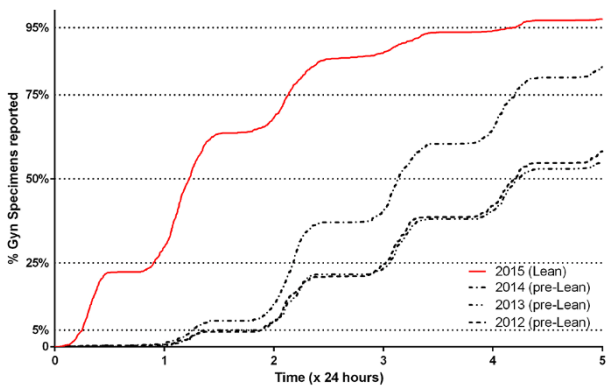
Steven Goodman, Roxanne R Mody, Donna Coffey, Blythe Gorman, Eric Luna, Donna Armylagos, Mary R Schwartz, Dina R Mody, Yimin Ge. Houston Methodist Hospital, Houston, TX; University of Texas, Health Science Center, Houston, TX; BioReference Laboratories, Elmwood Park, NJ.
Background: High risk HPVs (hrHPV) are associated with the majority of cervical cancer and precancerous lesions. Published studies indicate preceding negative Pap tests may be seen in women with high grade cervical lesions (HGCL) on biopsy. This study aimed to determine the factors and HPV status that may contribute to the discordance.
Design: A total of 42,797 negative Pap tests (NILM) with HPV co-testing were identified from 171,621 Pap tests interpreted between March 1, 2013 and December 30, 2014. Follow-up biopsies were performed in 422 cases. Negative Pap tests with HGCL on biopsy were randomized with equal numbers of random cases from the study period for review by two cytopathologists blinded to the study.
Results: In this cytology negative cohort, HGCLs were identified in 22 (5.2%) of 422 biopsies with hrHPV positive in 20 (91%) and negative in 2 (9%). The HGCLs included 20 high grade squamous intraepithelial lesions (HSIL), 1 adenocarcinoma in situ and 1 squamous cell carcinoma. Positive hrHPV test was strongly associated with HGCL on biopsy ($p<.001$) with a high sensitivity (91%) and negative predictive value (NPV, 99%), but low specificity (45%) and positive predictive value (PPV, 8%). Upon review of the available 21/22 NILM Paps, 9 (43%) were upgraded to ASCUS or higher, including 3 (14%) HSIL or ASC-H. All upgraded cases were hrHPV positive. Discrepancies for minor lesions were observed between reviewers in two cases. The contributing factors for negative Pap tests were lack of abnormal cells (10/21, 48%), low grade cells not diagnostic for HGCL (6/21, 28%), unsatisfactory sample (2/21, 10%), and interpretation variances due to marked inflammation (3/21, 14%).
Conclusions: Negative Pap tests were identified in 5.2% of women with HGCL on follow-up biopsy in this low risk population. Although hrHPV was detected in most of the HGCL cases, its specificity for HGCL was low (45%). As many as 92% of the hrHPV-positive cases were negative for HGCL on biopsy, resulting in a low positive predictive value (PPV) for HGCL by either hrHPV test (8%) or HPV 16/18 genotyping (11%). The most common reason for negative Pap tests in women with biopsy-confirmed HGCL was absence of diagnostic cells (86%), presumably due to sampling variance. Obscuring inflammation was the main contributing factor for interpretation variance. Negative Pap tests with marked inflammation and positive hrHPV may warrant rescreening to minimize the false-negative rate.

1971 Daily Surgical Pathology Huddles Positively Impact Quality, Safety Culture and Team Engagement

Omar Hameed, Cheryl M Coffin, Anatomic Pathology Team. Vanderbilt University Medical Center, Nashville, TN.
Background: High reliability practices are significantly associated with improved quality and patient safety in a variety of healthcare settings. For example, timeouts (team huddles) at the start of surgical procedures is a high reliability practice that, because of its positive impact on patient safety, has become the standard of care. This study describes the implementation and impact of such a team huddle in surgical pathology as part of systematic effort to expand high reliability practices and improve safety culture across the anatomic pathology lab of a tertiary academic medical center.
Design: Results of a standardized patient culture survey along with additional systematic collected data and gap analyses were used to develop specific and focused quality improvement (QI) projects. This included implementation of a daily surgical pathology huddle. The impact of this on different quality metrics, safety culture and team engagement was assessed.
Results: The entire gross room/frozen section (FS) team for day participates in the huddle, including pathology technologists, pathologists' assistants, residents rotating in surgical pathology, the surgical pathology fellow and attending pathologist. Main items that are communicated in this approximately 5 min briefing/debriefing session are highlights of the operating room schedule including predicted intraoperative consultations, key volume metrics for the previous day, staffing coverage, expected slide delivery times from histology and debriefing of previous day work issues. For continuous improvement, a survey was performed that indicated that most participants thought that the huddle provides up-to-date, easy-to-follow information, addresses relevant questions, clarifies issues and helps better prepare for the day. The impact of this huddle and implementation of other associated high reliability practices included (1) reduction of major FS discrepancy rates, (2) reduction of single and multiple FS TAT, (3) increased number of QI projects across the lab, (4) increased engagement at all levels and (5) improvement in the saftey culture. The latter was manifested by improvements in 9 of 12 safety dimensions measured in a repeat survey, including a 5% improvement in the overall perception of safety.
Conclusions: Huddles in surgical pathology provide a constructive, blame-free environment for respectful personal interaction that allows for dissemination of information and brief discussion, create a nidus for generating additional improvements and result in improved outcomes and increased engagement and mindfulness of the entire team.

1972 Implemenation of a "Lean" Cytopathology Service - Towards Routine Same-Day Reporting

Ekkehard Hewer, Anja M Schmitt. University of Bern, Bern, Switzerland.
Background: Lean management has gained popularity for design of faster and safer processes in health care. A continuous workflow as opposed to processing of large batches of specimens is central in lean management and makes it particularly suitable for cytopathology. Nevertheless, only few studies adress the effect of lean management principles with respect to organiaziation of diagnostic cytopathology units.
Design: We applied lean management principles to reorganize our diagnostic cytopathology service. Changes included introduction of a continuous workflow (facilitated by modernization of key devices) and a structured electronic reporting system. We monitored the effects of these changes on turnaround times.
Results: Introduction of a fully "lean" workflow for cervical cytology increased the percentage of specimens reported within one week from 65% to 97%. 63% of cervical cytology specimens were reported within one working day as opposed to 6% at baseline.



Continuous processing of non-gynecological specimens alone (without changing the reporting process) resulted in 18% and 77% of cases being reported on the same day or within one working day, respectively (3% and 55% pre-Lean).
Conclusions: Stringent application of Lean Management principles allows for significant reductions in turnaround-times. It may therefore contribute to routine same-day reporting of cytological specimens.

1973 Validation of Digital Whole Slide Imaging System for Intraoperative Breast Sentinel Lymph Node Touch Prep Analysis: A Single Institution Experience

Jenny Hoffmann, Lisa McGinnis, Chrisy T Mafnas, Jennifer Ziskin, Ann K Folkins, Kimberly Allison, Robert B West, John P Higgins, Neeraja Kambham, Steven R Long, Brent Tan. Stanford University Medical Center, Stanford, CA.

Background: Whole slide imaging (WSI) is an emerging technology with multiple potential applications, including diagnostic, educational, archival, and research purposes. The role of WSI in the evaluation of breast sentinel lymph node touch preps has yet to be described.

Design: A retrospective search of the surgical pathology database of Stanford University Medical Center identified 50 intraoperative touch prep slides, each representing a distinct sentinel lymph node biopsied for intraoperative consultation from 26 patients with invasive ductal carcinoma and/or ductal carcinoma in situ. Glass slides were screened to confirm adequacy for scanning; 2 slides were excluded due to too few cells or technical issues with scanning. The remaining 48 slides were scanned at 200x magnification on an Aperio ScanScope CS2. Each scanned slide was then reviewed a second time by the pathologist who originally reviewed the glass slide at the time of intraoperative consultation. Slides were scored as positive, negative, or defer.

Results: Of the 11 lymph nodes that originally interpreted as involved by metastatic carcinoma on glass slides, review of the corresponding WSI yielded 7 interpreted as positive, 2 interpreted as defer, and 2 were interpreted as negative. In the 2 cases where WSI yielded a "false negative" result, the malignant cells were focal. Of the 35 lymph nodes which were interpreted as negative by glass slide evaluation, 34 slides were interpreted as negative by WSI. One slide was interpreted as negative by one reviewer and atypical by the second reviewer in the remaining case. The kappa value for intra-observer reproducibility was 0.66 (0.4-0.91). Of the cases which were classified atypical/defer by WSI, reasons for deferral included the preparation being too thick and obscuring air bubbles.

Conclusions: Intraobserver reproducibility was fair to good when comparing glass slides and WSI interpretation. A greater degree of deferral rate was seen when using WSI, and 2 cases which were interpreted as positive by glass slide interpretation was read out as negative by WSI. While WSI is a promising technology, additional studies need to be performed to evaluate the efficacy and safety of its use in a clinical setting for sentinel lymph node touch prep evaluation.

1974 A Triaging Strategy Using Absolute White Blood Cell Differential Counts and Smear Morphology Improves Diagnostic Yield of Peripheral Blood Flow Cytometry

Amelia Huck, Christine Hong, Michelle E DeLelys, Frederic I Preffer, Aliyah R Sohani. St. Barnabas Medical Center, Livingston, NJ; Harvard College, Cambridge, MA; Massachusetts General Hospital, Boston, MA.

Background: Peripheral blood (PB) flow cytometry (FC) is a useful tool for evaluating circulating hematologic malignancies (HM), but is often negative when used as a screening test. We previously demonstrated that a triaging algorithm using history of HM and cerebrospinal fluid (CSF) white blood cell (WBC) counts and morphology improves CSF FC diagnostic yield without loss of clinically important data (Kovach et al. *Am J Hematol* 2014). We evaluated whether a similar strategy using PB WBC counts and smear morphology could be used to optimize PB FC utilization.

Design: We evaluated 349 consecutive PB samples submitted to our FC laboratory for testing with concurrent or recent (within prior 6 weeks) CBC/differential analysis. Cases were divided chronologically into test (n=155) and validation (n=194) cohorts to generate and apply triaging guidelines. FC results were classified as positive, negative or of limited clinical significance (LCS). Positive cases were classified as abnormal blast or lymphoid populations. Results were correlated with history of HM, CBC/differential and PB smear results.

Results: 119/159 samples (75%) from patients with a history of HM and 42/190 (22%) from patients without prior HM had positive FC results. In patients without prior HM, high absolute lymphocyte count (ALC) and abnormal cells on PB smear were associated with positive FC results, while normal absolute WBC differential counts (including an isolated high absolute neutrophil count [ANC]) in the absence of abnormal circulating cells was associated with negative or LCS FC results (Table 1). Deferral of FC analysis on the latter group would have resulted in testing 19% fewer cases without a negative impact on clinical care: 6 LCS cases (all monoclonal B-cell lymphocytosis, none of which were treated) would have been missed, but no other abnormalities would have gone undiagnosed.

FC Result	Blast	Lymphoid	LCS	Negative	p-value[1]
History of HM	29	90	0	40	<0.0001
No History of HM	26	16	18	130	
High ALC	5	14	5	8	<0.0001
Abnormal Circulating Cells[2]	18	3	3	3	<0.0001
Normal absolute WBC counts[3] and no abnormal circulating cells	0	0	6	59	<0.0001

Conclusions: Our findings support a triaging strategy using HM history, absolute WBC differential counts and smear morphology to improve the diagnostic yield of PB FC and reduce unnecessary use of a costly test.

1975 Impact of Changes in the Helicobacter Pylori (HP) Detection Protocol on Reported HP Rates

Bogdan Isaila, Razvan Lapadat, Yi Zhou, Xianzhong Ding, Stefan Pambuccian. Loyola University Medical Center, Maywood, IL.

Background: Helicobacter pylori (HP) is involved in the development of various upper gastrointestinal tract conditions, including chronic gastritis, gastric and duodenal ulcers, and gastric carcinoma and lymphoma. Protocols for its detection in gastric biopsies vary substantially between laboratories, with some laboratories using immunostains or histochemical stains either routinely or depending on the histologic findings. The current guidelines recommended the use of HP immunohistochemistry as the preferred ancillary staining method only when biopsies show chronic, or chronic active gastritis without detectable HP in H&E-stained sections. The aim of this study was to determine the impact of changing the HP detection protocol from routine use of ancillary stains (Giemsa) to "on demand" use of (HP immunostains) on the HP reporting rate.

Design: Our electronic database was searched for all gastric biopsies reported from 9/22/2010 to 9/21/2015; data regarding the presence of HP and ancillary stains were extracted for the period before (P1) and after (P2) the implementation of the change in HP detection protocol (4/15/2014). The detection frequency of HP and the frequency of use of HP immunostains was compared for P1 and P2 using chi-square tests. The HP primary antibody used is polyclonal rabbit (Cell Marque 215A-78).

Results: 13,363 gastric biopsies were reported during the study interval; 9156 in P1 and 4207 in P2. The HP detection rate was 10.75% (334/3107) for 2010-2011, 10.13% (606/5985) for 2012-4/15/2014 and 8.84% (372/4207). The difference between the HP detection rate in P1 (10.3% (943/9156)) and P2 (8.84% (372/4207)) was significant ($p<0.01$) and did not appear to be related to temporal trends. However, a change in patient population cannot be ruled out, since our institution expanded its GI services. The rate of HP immunostain use in P2 was 39.62% (1667/4207).

Conclusions: Despite the use of HP immunostains on a rather high percentage of gastric biopsies, we found a drop in the reported prevalence of HP after discontinuing "up front" testing for HP with Giemsa stains. These findings could be due to changes in the HP infection rate in the population served due to an increased diagnostic specificity due to less artifactual interpretation of Giemsa stains. Monitoring HP reporting rates and correlating histologic and IHC positivity with the HP detection methods could be useful as a quality assurance tool.

1976 The Utility of Intraoperative Gross Examination of Colorectal Resections: A Retrospective Review of 200 Sequential Cases

Armen Kharajian, Ajuni Choudhary, Alexander Baras. Johns Hopkins, Baltimore, MD.

Background: Intraoperative pathology consultation (IPC) is an important tool for many surgical procedures and is generally deemed appropriate when the result immediately alters management. Two types of IPC exist: frozen sections and gross exam only. Gross exams identify lesions and measure the distance to nearest margin. Our goal was to evaluate the utility of intraoperative gross examination (IGE) of colorectal resections and their effect on surgical management.

Design: The pathology database of our institution was searched for cases in which an IGE for colorectal resection was performed and filtered based on whether an extra specimen was received that could have been due to the findings reported. Operative notes were reviewed to determine whether the extra specimen was due to the IGE.

Results: 200 cases over a 14 month time-frame were reviewed. 21 different final diagnoses were rendered. The 3 most common accounted for 78% of cases; adenocarcinoma (95), IBD (42), and diverticular disease (19). 14 surgeons requested IGE with the top 6 accounting for 91% of cases. After thorough review of the operative notes, none of the 200 IGE cases had additional tissue resected as a direct result of the IGE findings. Additionally, we found no statistically significant difference in the distance to closest margin when comparing IGE to a set of control cases with no intraoperative consultation.

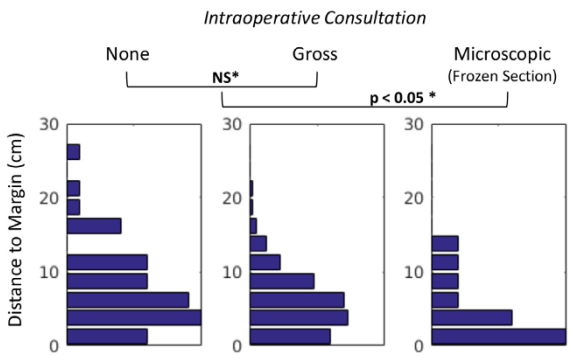


Figure 1. The distribution of distance to nearest margin stratified by the type of intraoperative consultation for neoplasms in the colon.
The histograms of distance to nearest margin via horizontal bar graphs are shown for none, gross, and microscopic intraoperative consultations (as designated above). No significant difference (NS) was observed when comparing gross only to no intraoperative consultation. Cases with frozen sections were significantly enriched for smaller distances to nearest margin.
*Kolmogorov-Smirnov test of the distributions and Fisher's Exact test for distance < 5 cm.

Conclusions: Surgeons request IPCs for many reasons including necessity for immediate management, research interests, and providing patients immediate results. However,

IPCs should only be used when results will immediately alter surgical management. Our study showed that no additional surgical management was undertaken given the results from IGE. The most applicable use of IGE is margin status evaluation, and the most recent NCI guidelines recommend five cm of clearance. Extra margins were not received for the cases with less than five cm of clearance and no difference was observed when comparing to control cases with no IPC. As we continue to battle rising health care expenses, ways to reduce costs will be crucial. Decreasing unnecessary intraoperative consultations may represent a cost and resource saving measure in surgical pathology.

1977 Is Digital Imaging a Viable Method of Measurement for Breslow Thickness in Invasive Melanomas? An Example of Evaluation of Uncertainty in a Critical Value in Cellular Pathology

Yi Ling Khaw, Barbara Loftus. Tallaght Hospital, Dublin, Ireland.

Background: In cellular pathology, the assessment of the uncertainty of measurement (UoM) is an unfamiliar area of quality assurance. The Royal College of Pathologists UK (RCPath) issued a set of guidelines in May 2015 to outline the most appropriate ways to meet this aspect of the ISO 15189 standard. Breslow thickness (BT) is a critical value which determines stage, prognosis and plays a major role in patient management. Therefore it is appropriate to consider the uncertainty of its measurement. In this study, we also determine if Digital Imaging (DI) is a viable alternative modality of measurement in addition to the Vernier Scale (VS).

Design: OBJECTIVES: (i) To measure precision / UoM. (ii) To determine if DI is a viable alternative to the VS (intermodality variability). (iii) To determine baseline interobserver and intraobserver variability for future audits. METHOD: Glass slides for 50 cases of cutaneous invasive melanoma were examined. BT was re-measured by the Reviewer using both modalities VS and DI. These values were compared with the Reported BT values.

Results: The **mean variation** was **0.078mm** and this value is taken to represent **overall precision**. In our opinion, this value is chosen to best represent **UoM**. Interobserver, intraobserver and intermodality variations (each of the three groups) were not **statistically significant**. When the DI and VS (intermodality variation) were compared, the resulting measurements were extremely precise with a difference of only **0.064mm**. This value has been used as a surrogate marker for **intraobserver variation** and thus reflects good intraobserver reproducibility.

Conclusions: Measurements with both DI and VS are both **precise and very reproducible**. We conclude that DI is a more useful when a **second decimal point** is mandatory in situations when it is likely to change the pT stage ie. at the pT stage boundaries. DI should be recommended for use as per personal preference. The estimated **uncertainty of measurement** is small 0.078mm. This value will be used in future audits. In order to minimise intra- and interobserver variation, standard operating procedure (SOP) for the process of measuring BT will be created.

1978 Quality Assurance of Anatomic Pathology Diagnoses: Comparison of Alternate Approaches and Detection of Diagnostic Error

Lester Layfield. University of Missouri, Columbia, MO.

Background: Traditionally, a 10% review has been the basis for quality assurance programs in anatomic pathology. The effectiveness of such a protocol has been questioned and alternative methodologies suggested. Little published data exists comparing yield for detection of diagnostic errors by alternate strategies.

Design: The detection rate for diagnostic errors in surgical pathology was compared over a three-month period using different review procedures comprising: random 10% review, correlation of internal and external diagnoses following solicited external expert opinion, a focused review of dermatopathology cases and correlation of internal diagnoses with outside diagnoses in cases sent for review at a second institution treating the patient. Error rate was expressed as percentage of reviewed cases where the initial diagnosis differed from the review diagnosis. Error rates detected by each method were compared between the methods.

Results: The 10% random review detected six errors in 597 cases (1%). Solicited case consultations requested by clinicians or internal pathologists detected two diagnostic errors in 16 cases (12.5%). Unsolicited reviews by outside institutions in course of patient care detected one diagnostic error in 46 cases (2%). Review of the dermatopathology material disclosed 5 diagnostic errors in 59 cases (8.5%).

Conclusions: The alternate review procedures have different biases which appear to result in different frequencies of detected error. The 10% random review includes larger numbers of routine cases (appendectomies, etc.) where there is little chance for diagnostic error. The other methodologies are more likely to review diagnostically problematic cases associated with a greater number of errors. Cases referred to expert consultants for diagnostic concerns would be expected to have the highest rate of error as demonstrated in the present study (12.5%). Cases sent for review associated with treatment at a second institution represent therapeutically important specimens but are less likely to be selected due to questions concerning the original diagnosis. Focused reviews initiated by diagnostic concerns of a clinician or pathologist, unsolicited reviews because of treatment at another institution and sub-specialty based reviews appear to be more effective in detecting diagnostic errors than the standard 10% random review. Quality assurance programs should include focused reviews in addition to 10% random review to maximize error detection.

1979 Ancillary Testing in Bone Marrow Staging for Lymphoma: A Laboratory Utilization and Quality Management Opportunity

K David Li, Jerry W Hussong, Sherrie L Perkins, Jay L Patel. University of Utah/ARUP Laboratories, Salt Lake City, UT.

Background: A diagnosis of lymphoma is typically followed by bone marrow staging with associated ordering of extensive ancillary testing at the time of procedure. This practice presents a utilization management challenge since morphology in conjunction with limited immunophenotyping remains the diagnostic benchmark. We hypothesize the majority of such testing is noncontributory to patient management and represents an opportunity for cost savings without impact on the quality of care.

Design: We conducted a search of our information system for all adult bone marrow samples submitted to our laboratory in the past 5 years with a specified indication of initial lymphoma staging. This yielded a total of 227 patients (M:F ratio, 1.3; age range, 20-90; median, 58 years). Pathology reports were reviewed and data extracted including primary diagnosis, staging result and status of ancillary studies. Cost analysis was performed using a test-specific 12-month average price history.

Results: :

Diagnosis	Number (%)	Marrow Involved (%)
Hodgkin		
Classical Hodgkin	40 (18)	5 (13)
Nodular lymphocyte predominant Hodgkin	2 (1)	0 (0)
B-cell		
Diffuse large B-cell	85 (37)	11 (13)
Follicular	35 (16)	15 (41)
Mantle cell	15 (7)	10 (67)
Burkitt	5 (2)	1 (20)
Other B-cell NHL	35 (15)	8 (23)
T-cell		
Angioimmunoblastic T-cell	3 (1)	2 (67)
Peripheral T-cell	3 (1)	1 (33)
Other T-cell NHL	4 (2)	0 (0)
Total	**227 (100)**	**53(23)**

Study Performed	Number of cases (%)	Positive (%)	Negative (%)	Sensitivity (%)	Specificity (%)	Average cost per unit ($)
Morphology (core and clot)	227 (100)	44 (20)	183 (80)	83	100	140
Flow cytometry (22 markers)	206 (91)	38 (18)	168 (82)	77	96	447
Karyotype	144 (63)	13 (9)	131 (91)	38	100	396
FISH (3 probes)	23 (10)	2 (9)	21 (91)	38	100	550

Conclusions: The diagnostic performance of morphologic evaluation in the context of lymphoma staging is high and when combined with flow cytometry, sensitivity exceeds 98%. Genetic studies (karyotype and FISH) are highly specific when lymphoma-associated abnormalities are found, but demonstrate low yield while contributing significant cost. We noted only one instance of a positive karyotype finding in the absence of morphologic and immunophenotypic evidence of lymphoma. These data support implementation of a decision tree approach to ancillary testing which begins with morphology followed by limited immunophenotyping (by flow cytometry or IHC). Genetic studies should be deferred (culture, nucleic acid extraction and hold) and can be performed subsequently if indicated. The mean cost per marrow is $851 given observed utilization. Conservative modeling suggests at least $305 per specimen could be saved by implementing a stepwise testing strategy.

1980 Evaluating the Immunostaining Detection of Cytomegalovirus in Gastrointestinal Biopsies: Clinical Pathological Correlation

Xiaoyan Liao, Grace Y Lin. UCSD, San Diego, CA.

Background: Cytomegalovirus (CMV) infection is ubiquitous and can be subclinial and asymptomatic. At our institution, immunostain for CMV is ordered if clinically warranted and ulceration present. We aim to study the clinical-pathological correlation of CMV immunostaining detection in gastrointestinal (GI) biopsies to provide a practical guideline for better management.

Design: Pathology information system was electronically searched, and 935 out of 7421 total non-neoplastic GI biopsies (12.5%) were tested for CMV between 1/1/2013 – 6/30/2015. Ninety-one (1%) cases reported positive stains and were re-evaluated microscopically. The results are classified as "positive" if >2 cells are stained (n=49), or "rare positive" if 1-2 cells are stained (n=42). Of these 91 cases, pre-existing conditions include inflammatory bowel disease (IBD, n=34), HIV infection or AIDS (n=15), and other immunocompromised conditions including solid organ or bone marrow transplant (n=42). Clinical information (virology tests and specific treatment) and follow-up biopsies are reviewed. Statistical analysis is performed using SPSS.

Results: Overall 1% of GI biopsies show immunostaining for CMV. The more tests ordered, the smaller percentage of cases were detected. Viral cytopathic effect is specific but not sensitive as only 9 (10%) cases were reported. Clinician's request to rule out CMV infection yields higher detection rate (43/118, 36%) than overall (91/935, 9.7%). The most commonly involved organ is colon, followed by esophagus, stomach, ileum and duodenum. When >2 cells are detected, CMV virology is positive in 55%, negative in 16%, not tested in 28% of cases. When only 1-2 cells are detected, CMV virology is positive in 24%, negative in 24%, not tested in 52% of cases. The difference is statistically significant by Fisher's exact test ($p<0.01$). Follow-up biopsies in CMV "rare positive" patients are frequently negative, and only 31% of those patients are treated comparing to 71% treated in CMV "positive, >2 cells" patients (Chi-square=14.8, $p<0.001$). However, BMT patients are more likely to have positive virology results even if only rare cells are detected, and patients with HIV/AIDS are usually treated for CMV regardless of number of cells detected.

Conclusions: Clinical suspicion for CMV infection and viral cytopathic effect if observed, are indications for ordering immunostains. CMV virology correlates well with positive immunostains (>2 cells). Rare positivity is clinically significant in BMT and HIV/AIDS patients, but is usually not considered significant in IBD patients.

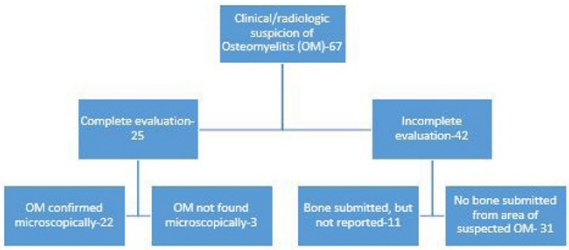
1981 Osteomyelitis and Lower Extremity Amputations at a Single Institution: A Quality Assurance Study

Valerie Lockhart, Erin E Langford, Guillermo A Herrera. LSU Health Shreveport, Shreveport, LA.

Background: The diagnosis and treatment of osteomyelitis (OM) requires a multi-disciplinary approach. Establishing an accurate diagnosis of OM is imperative, since treatment with prolonged antibiotic therapy and/or aggressive surgical intervention is necessary. Bone biopsy, the gold standard for diagnosing OM, is rarely done. X-ray and MRI are the imaging modalities of choice for diagnosis of OM. Without pathologic confirmation of suspected OM, radiologists/surgeons will lack quality assurance information with which to improve patient care.

Design: 437 lower extremity amputations received at our institution from 2011-2015 were identified in Co-Path. EPIC records were reviewed to identify pre-amputation clinical/radiologic suspicion for OM, and 67 cases were found. The cases were stratified into two groups based on radiology and pathology reports: incomplete and complete evaluation for OM. Incomplete evaluation was subclassified into bone submitted (but not reported on final diagnosis) and no bone submitted from area of suspected OM. Complete evaluation was subclassified into OM found/not found microscopically.

Results:



Of 67 specimens with suspected OM, 25 (37.3%) were completely evaluated and 42 (62.7%) were incompletely evaluated for OM. Of the completely evaluated specimens, OM was microscopically confirmed in 22 cases (88%). The remaining 3 cases (12%) showed no microscopic OM. Of the specimens incompletely evaluated for OM, bone was not submitted from the area of suspected OM in 31 cases (73.8%), and bone was submitted but results were not reported in 11 cases (26.2%).

Conclusions: OM was diagnosed in only 22/67 (32.8%) cases of suspected OM over 5 years, due to inadequate sampling/evaluation of lower extremity amputation specimens. Our results suggest inadequate grossing of lower extremity specimens. Bone should be submitted from every lower extremity specimen with clinical or radiologic suspicion for OM, and we should not rely on the requisition form alone to identify suspicion for OM. By identifying this deficiency in our practice, we can educate all resident and attending pathologists and improve our evaluation of lower extremity amputation specimens.

1982 The Impact of Subspecialty Surgical Pathology on Generalized Intraoperative Consultations

Sophia Ma, Ketan Patel, Kumarasen Cooper. Hospital of the University of Pennsylvania, Philadelphia, PA.

Background: Surgical pathology has over the past 20 years undergone a move towards subspecialization, as expertise becomes essential in this era of health care teams. At the Hospital of the University of Pennsylvania, the transition to subspecialty surgical pathology sign out occurred in 2009. Nevertheless, as in most subspecialized practices, the intraoperative consultation or frozen sections are in the domain of generalized surgical pathology. The surgical pathologist, though subspecialized in final signout, retain competency in a generalized environment at the frozen bench. The quality assurance of frozen section to final diagnosis correlation impact of this dichotomy has not been fully addressed.

Design: A quality assurance review of frozen vs final diagnostic correlation was performed comparing two twelve-month span of specimens requiring intraoperative diagnoses: 1099 specimens from July 2008 to July 2009 and 2908 specimens from July 2014 to July 2015. The discordant cases were then reviewed to determine type of discordance, either interpretive or sampling, and specimen type. Medical significance (no, minor, or major) was determined at the time of final diagnosis.

Results: We identified 29 (2.64%, range of 0% to 6.78%) discordant correlations in 2008-2009 and 60 (2.06%, range of 0.77% to 4.04%) in 2014-15 without a significant difference between the two years (p=.22). The disagreements were further characterized by sampling or interpretive discrepancies. 62% in 2008-09 and 68% in 2014-15 of errors were interpretive discrepancies in both years. The organ systems represented in greatest numbers of are head and neck (34% in 2008-09 and 43% in 2014-15), gastrointestinal (14% and 30%), male and female genitourinary (28% and 8%), and pulmonary (14% and 7%). In 2008-09, 13.7% and in 2014-15, 13.3% of discordant correlations had major medical significance.

Conclusions: Comparing the frozen section to final diagnosis, the current year did not show a significant increase in discrepancies or in major patient harm even though subspecialty surgical pathologists were faced with a variety of general intraoperative specimens. However, most of the senior attendings had trained and practiced in a generalized surgical pathology setting, which may skew the findings. Somewhat interestingly, the subspecialization seemed to foster an environment of collaboration and consultation among the various experts the department. Still, it is critical for surgical pathologists-in-training to retain a broad general surgical knowledge base.

1983 Impact of 2013 ASCO/CAP Guideline Recommendations on HER2 FISH Testing in Breast Cancer

Shivali Marketkar, Kamaljeet Singh, Cynthia Jackson, Jesse Hart, Murray B Resnick, Yihong Wang. Rhode Island Hospital, Providence, RI; Women and Infants Hospital, Providence, RI.

Background: The College of American Pathologists/American Society of Clinical Oncology (CAP/ASCO) guideline recommendations for Human Epidermal Growth Factor Receptor 2 (HER2) Fluorescent in situ Hybridization (FISH) testing for breast cancer were updated in 2013. They lowered the threshold for HER2 FISH positivity to HER2/CEP 17≥ 2.0. Average HER2 copy # ≥6.0 are considered positive, whereas HER2 copy # ≥4.0 to < 6.0 and HER2/CEP17 <2.0 are considered equivocal

Design: We conducted a two-center review of breast carcinomas with HER2 FISH results to assess the impact of updated guidelines. We included all invasive breast carcinomas during the 18 months before (06/2012–12/2013) and after (1/2014-06/2015) HER2 guideline updates implemented. We performed immunohistochemistry (IHC) for HER2 as the primary assay to identify HER2 amplification. All HER2 equivocal (2+) cases by IHC are reflexed to FISH testing. The results of HER2 FISH positive (HER2+), equivocal (HER2e), and negative (HER2-) cases were interpreted by the guidelines in place at the time of testing.

Results: A total of 1212 cases were included in the study. 91.4% were women. 88.3% were primary breast carcinomas and 11.7% metastases. The common metastatic sites were liver (16.2%) and bone (32.4%).The distribution of HER2 FISH results before and after the guideline updates are listed below.

	6/2012 to 12/2013	01/2014to 6/2015	p value
Total cases	562	650	
Her 2+	49(8.7%)	47(7.2%)	0.3965
Her2 -	490(87.2%)	559(86.2%)	0.8991
Her 2e	18(3.2%)	38(5.8%)	0.0397*

The number of cases reflexed to HER2 FISH testing increased by 92 (7.6%) by 2013 ASCO/CAP IHC criteria in the 18 month period after implementation. After the updated guidelines, the number of HER2e results increased significantly from 3.2% (18) to 5.8% (38), *p<0.05. When the 18 HER2e cases were re-evaluated according to 2013 criteria, 12 would have been reclassified as HER2+, whereas 6 remained equivocal. Of the 38 HER2e cases using 2013 criteria, 31 would have been classified as HER2- according to the 2007 criteria; 7 would have remained HER2e. 11 cases were insufficient.

Conclusions: Evaluating samples using 2013 ASCO/CAP criteria has increased the number of FISH reflex tests and HER2 positive cases allowing more patients with breast cancer to be eligible for HER2 targeted therapy, potentially improving their outcome. However, HER2e increased significantly using 2013 guidelines, posing a challenge for clinical management.

1984 Cost Effectiveness Analysis of Core Needle Biopsy Diagnosis of Papillomatous Breast Lesions

Clare McCormick-Baw, Mae Lopez, Ada Werlang-Perurena, Riyam T Zreik, Amin Mohammad, Arundhati Rao. Baylor Scott and White Memorial Hospital, Temple, TX.

Background: Papillary lesions of the breast presenting with mammographic abnormalities or nipple discharge can be diagnosed as benign, atypical or malignant on core needle biopsies (CNB). Subsequent treatment algorithms can range from routine screening to surgical excision resulting in significantly differing healthcare costs. In this study, we present a cost-effectiveness analysis that determines the value of immunohistochemistry (IHC) aided definitive diagnosis using CK5/6 and p63 on downstream management of breast papillary lesions.

Design: The surgical pathology database, at our institution, was queried for papillary lesions of the breast. Cases queried spanned a five year period with a minimum of 3 years follow up (n = 140). Morphology, p63 and CK5/6 IHC status was reviewed. Cost analysis using fiscal year (FY) 2015 CPT codes was performed after reviewing clinical history and treatment.

Results: Of the 140 cases evaluated, 115 were diagnosed as benign on biopsy whereas 25 were diagnosed as atypical or malignant. 107 patients underwent surgical excision and 63 cases were diagnosed benign on both the NCB and excision. 19 cases were diagnosed as atypical on excision, previously diagnosed as benign on CNB. CK5/6 and p63 staining of the 19 benign NCBs identified 17 as atypical. IHC also identified all atypical lesions diagnosed by morphology alone (n=22). The sensitivity of using IHC, in addition to morphologic analysis, for a resection confirmed diagnosis was 95% versus 54%. Analysis of applicable FY2015 CPT coding shows the cost of biopsy with follow up surgical excision is $2047.65 per case. Performing IHC on the initial CNB would cost $416.48 per case. The total cost of the 107 cases that had both biopsy and follow up surgery is $219,098.55. When IHC staining was used, the cost of 107 biopsies is $44,563.36. However, the savings for benign lesions not requiring surgery is $115,567.40.

Conclusions: The high sensitivity and specificity of p63 and CK5/6 IHC staining in papillomatous lesions of the breast, resulting in reliable differentiation of benign from atypical lesions, should lead to more cost effective treatment algorithms sparing unnecessary surgery. This analysis is conservative and further analysis including cost of complications and morbidity are being performed. The analysis may be further refined after additional screening costs resulting from the five-fold increased risk of malignancy in patients with these lesions are included.

1985 Bone Marrow Biopsy Practice and Quality Trends in USA and Canada: A Multicenter Study

Mihai Merzianu. Roswell Park Cancer Institute, Buffalo, NY.

Background: Bone marrow biopsy (BMB) is essential for diagnosis of various hematologic disorders. We studied BMB clinical practice and its quality assessment (QA) in US and Canada.

Design: Data were collected from 100 consecutive BMB samples/year/center for 2001 and 2011, and through a survey at 32 teaching hospitals from Northeast (n=10), Midwest (n=7), South (n=6), West (n=6) and Canada (n=3). Postprocessing core biopsy length (CL) for each BMB was uniformly measured at each site with a validated method and 6374 samples accepted for statistical analysis (SAS, Cary, NC).

Results: Most common BMB indications were acute leukemia/myeloid neoplasms (42%) and lymphoma staging (19%); 44% for initial workup and 52% for follow-up. BMB was performed by hematologists (59%), pathologists (8%) and non-physicians (31%; increased by 50% from 2001 to 2011), with respective CL yield of 13.7 mm, 21.4 mm and 12.6 mm (p=0.038).

Bedside pathology BMB assistance was common in 64%, rarely or never done in 36% of centers, with respective CL of 14.8 and 12.7 mm (NS). Bilateral BMB (7%) decreased by 62% from 2001 to 2011. Aspirate smears, clot sections, touch imprints, CBC data and blood film for review were available in 86%, 56%, 66%, 78% and 71% cases, respectively.

Common fixatives were buffered formalin and heavy metal fixatives (HMF)(34% each), zinc-based formalin (ZBF; 16%) and Bouin's (11%). Usage of ZBF increased (6 to 25%) whereas HMF decreased (53% to 16%) from 2001 to 2011. Routine studies included Wright Giemsa (62%) and iron (44%) for smears, iron (30%), PAS (16%), Giemsa (12%) and reticulin (11%) for clot and/or core.

All ancillary studies (AS) use increased from 2001 to 2011: flow cytometry (35% to 71%), cytogenetics (38% to 71%), immunohistochemistry (20% to 45%) and molecular studies (5% to 21%) (all p<0.001). The annual BMB volume was ≥1000 in 43% and <1000 in 56% of centers with respective CL of 14.8 mm and 13.2 mm (p=0.014). BMBQA was present in the reports from 58% of centers but only 36% of sites had an institutional QA (IQA) program.

Overall CL averaged 14.1 mm, was ≥15 mm in 38% and ≥20 mm in 19% of all BMB, and ≥25 mm in 15% of lymphoma staging BMB.

Conclusions: Several BMB practice changes occurred in the past decade: non-physician operators and AS usage increased from 2001 and 2011. IQA was absent in most and BMBQA was not documented in almost half of the centers. Pathology personnel participation in BMB and higher volume correlated with longer cores. Current recommendations for optimal core length (15-25 mm) are met only in a minority of samples in routine clinical practice in academic centers.

1986 An iOS App to Expedite the Evaluation of Immunohistochemistry Stains

Wilfrido Mojica, Gregg Mojica. University at Buffalo, Buffalo, NY; Gradology, Inc, San Francisco, CA.

Background: Using tumor tissue to serve as control material for some antibodies is not possible based on the low frequency of some types of tumors and/or their dedication for use in research studies (e.g., mesothelioma for the calretinin antibody). Alternative tissues received at a greater frequency can then be used, provided it is made clear what cell type and cellular part(s) are expected to be stained (e.g., cytoplasmic and nuclear staining of peripheral nerves (ganglion cells and axons) in the appendix for the calretinin antibody). When this strategy is applied to numerous antibodies, recalling the expected staining pattern for every antibody may be difficult. As a means to help pathologists and technicians in our institution, a reference manual was initially created that delineated the expected staining patterns of the control tissues for each antibody, replete with images. Despite the presence of this tool, it soon became apparent that an alternative approach for the delivery of this information was necessary, one more mobile, less cumbersome and easier to navigate. An Apple-based Operating System (iOS) application was created to meet these new demands.

Design: Photomicrographs of control tissue used for each antibody were taken and embedded with explanatory text for each antibody. The data was then imported to a mongo db database powered by a Node.JS backend. A client side iOS application was then written in the new Swift 2.0 programming language. This app then downloads and manipulates the database's information in the appropriate format for either iPads or iPhones.

Results: The iOS app reduces the process of navigating antibody data in relation to control tissues to the movement or tap of a finger. Space is economized and mobility enhanced. Images can be easily expanded for further detail in the app when compared to the static nature in the traditional word or pdf format. Inclusion of new antibodies can be added to the database without the need to reprint an entire new volume. Links automatically take the viewer to the referenced item, not just the address.

Conclusions: The iOS format can be customized to fit the needs of any immunohistochemistry laboratory. The availability of reference data in this format simplifies the everyday quality control evaluation of each antibody and promotes accurate and reproducible assessment by participating pathologists. An android version may be needed to be developed in the future.

1987 Comparing H&E Stained and Unstained Slides of Frozen Tissue for Quality and Quantity of mRNA after Laser Capture Microdissection in Human Tissue for the Evaluation of the Microenvironment in Regressing and Progressing Primary Cutaneous Melanoma

Kumaran Mudaliar, Solomiya Grushchak, Arielle Gray, David Murray, Rebecca Tung, Stephanie Kliethermes, Michael I Nishimura, Kelli A Hutchens. Loyola University Chicago Stritch School of Medicine, Maywood, IL; Loyola University Medical Center, Maywood, IL.

Background: Up to 50% of primary cutaneous melanomas have areas of spontaneous tumor regression. This study attempts to compare the best method of slide preparation (unstained versus stained) prior to performing Laser Capture Microdissection (LCM) to isolate RNA for study of the subset of T lymphocytes found in areas of regression (RATs) from T lymphocytes found in areas of progressing tumor (TIL).

Design: Patient samples were collected and immediately frozen using precaution to limit the exposure of RNAses. Prior to LCM, 5-mm-thick frozen tissue sections were placed on uncharged glass slides. Samples were either left unstained or stained with a standard H&E protocol. LCM was performed immediately after slide preparation. Lymphocytes from the areas of regression, areas of progression, or non-specific inflammation were selected. RNA isolation was immediately performed after LCM. The quantity and quality (260/280 ratio) of the RNA was evaluated for stained slides compared to unstained slides.

Results: Overall RNA obtained from unstained slides was of equal quality compared to RNA from stained slides (Stained = 1.55, Unstained = 1.54). The overall quantity of RNA when compared to the stained slides (0.500 ug = median unstained, 0.432 ug = median stained) was more. Comparing the quantity and the quality of RNA did not yield significant results.

Figure 1

Tissue Sample	Amount (ug)	Quality (260/280 Ratio)
1	0.323	1.72
2	0.680	1.55
3	0.900	1.54
4	0.132	1.49
5	0.432	1.58
Median (min-max) Stained	0.432 (0.132-0.680)	1.55 (1.49-1.58)
Median (min-max) Unstained	0.500 (0.323-0.900)	1.54 (1.41-1.72)
P- value	0.75	0.99

Conclusions: Although there was no significant differences in the median quantity and quality likely due to the limited sample size, we feel the comparable RNA quality and the small increase in RNA quantity might be technically useful. The unstained slides did not hinder LCM extraction, and thus these results allow us to remove H&E staining from our methods. Less preparation results in less opportunity for RNA degradation. In addition, we plan to amplify the RNA in order to make quantity hurdles more surmountable.

1988 Comparison of UroVysion FISH and Urine Cytology with Performance Evaluation of Individual Chromosome Enumeration Probes: A Five Year Single Institutional Experience

Paari Murugan, Sandhya Dasaraju, Amrita Rao, Badrinath R Konety, Khalid Amin. University of Minnesota, Minneapolis, MN.

Background: The diagnosis and surveillance of urothelial carcinoma has traditionally been performed by cystoscopic biopsies and urine cytology. In the past decade, an ancillary technique that employs a multiprobe fluorescence *in situ* hybridization assay (UroVysion™ FISH) to detect chromosomal abnormalities in urothelial carcinoma has gained wide use and acceptance among urologists and cytologists as a screening as well as a diagnostic tool. In this study we present our 5-year institutional experience with the UroVysion™ FISH test.

Design: A total of 401 cases submitted for UroVysion™ FISH from 2010 to 2015 that had definitive cytology results available were included in the study. Various performance parameters of UroVysion™ FISH were evaluated in comparison with concurrent urine cytology. The FISH results were read

as positive if there were at least 4 nuclei with polysomy of 2 or more chromosomes 3, 7 and 17 in the same cell or a homozygous deletion of 9p21 was present in ≥12 cells. Additionally, in order to ascertain the performance value of each probe (CEP 3, 7 & 17) we compared them individually with the concurrent cytology findings. A cut-off value of four or more chromosome copy numbers averaged from five cells with the highest polysomies was considered as significant abnormality.

Results: Of the 401 cases, 70 were FISH positive and 87 showed positive cytology for high grade carcinoma. When compared to cytology, the sensitivity of FISH was 84% with positive predictive value (PPV) of 91%, and the specificity was 97% with negative predictive value (NPV) of 95%. The

performance parameters of individual chromosome probes showed that CEP 3 had sensitivity and PPV of 90% with specificity and NPV of 98%. The sensitivity and specificity of CEP 7 was 79% and 97% respectively (PPV of 87% and NPV of 96%) while CEP 17 had sensitivity of 70% and specificity of 99% (PPV of 93% and NPV of 94%).

Conclusions: We show that of the probes used in the UroVysion™FISH test, CEP 3 has greater sensitivity compared to CEP 7 and CEP 17. Nevertheless, all three

probes demonstrated high specificities. Overall, in our experience, UroVysion™ FISH demonstrated high specificity and acceptable sensitivity in detecting high-grade urothelial carcinoma.

1989 Validation of Computer-Assisted ER, PR, Her2, & Ki-67 IHC Quantitation

Charles Myers, Cynthia Cohen, Momin T Siddiqui, Bill Li, Godfrey M Guerzon, Diane Lawson, Geoffrey Smith. Emory, Atlanta, GA.

Background: Our laboratory uses a computer assisted quantitation methodology for interpretation of ER, PR, Her2, and Ki-67 on breast carcinomas. Our Dako Automated Cellular Imaging System III (ACIS) instrument is being replaced by a Leica Aperio AT2 (AT2). Validation consisted of a non-inferiority comparison of the AT2 to ACIS.

Design: Breast carcinomas from 2011-15 with ER, PR, Her2, and Ki-67 were reviewed. Equal numbers of positive and negative cases were selected, particularly around break points. Image analysis parameters were developed with a Leica consultant.

Result ranges for ER and PR are the same for ACIS and AT2: negative <1%; positive ≥1% nuclear positivity. Ki-67 ranges are also the same for ACIS and AT2: low <10%; intermediate ≥10% but <20%; high ≥20% nuclear positivity. Her2 ranges differ between the ACIS and AT2 due to algorithm outputs. The ACIS is based on a positive pixel count algorithm: negative/low 0-1.8; equivocal/intermediate 1.8-2.6; positive/high >2.6. The AT2 is based on a membranous staining categorization algorithm: negative/low ≥10% cells either 3+, 2+ and/or 1+; equivocal/intermediate ≥10% cells either 3+ and/or 2+; positive/high ≥10% cells 3+ (3+, 2+, and 1+ defined per Leica algorithm). Similar lesional areas were annotated and quantified on each instrument. After direct comparison, discordant cases were reviewed by pathologists for non-inferiority.

Results: Results Prior to Pathologist Review

	Negative	Negative Concordance	Equivocal	Equivocal Concordance	Positive	Positive Concordance
ER	13/14	93%	N/A	N/A	43/45	96%
PR	20/20	100%	N/A	N/A	35/37	95%
Her2	31/44	71%	72/74	97%	29/30	99%
Ki-67	9/9	100%	5/7	71%	8/9	89%

Pathologists assessed a discordant case as non-inferior if it would not affect a clinical decision. ER, PR, and Ki-67 were non-inferior if AT2 assigned a score near the break-point resulting in more intensive review. Her2 cases negative or positive by ACIS but equivocal by AT2 were non-inferior, as these reflex to FISH. Two equivocal cases by AT2, positive by ACIS were judged non-inferior due to concordance by FISH.

Concordance after Pathologist Review

	Negative	Equivocal	Positive	Total
ER	100%	N/A	100%	100%
PR	100%	N/A	100%	100%
Her2	100%	100%	100%	100%
Ki-67	100%	100%	89%	96%

Conclusions: The ACIS and AT2 quantitation algorithms for ER, PR, Her2, and Ki-67 produce similar results when the same regions of interest are analyzed. Using our computer assisted quantitation methodology, the AT2 is not inferior to the ACIS platform.

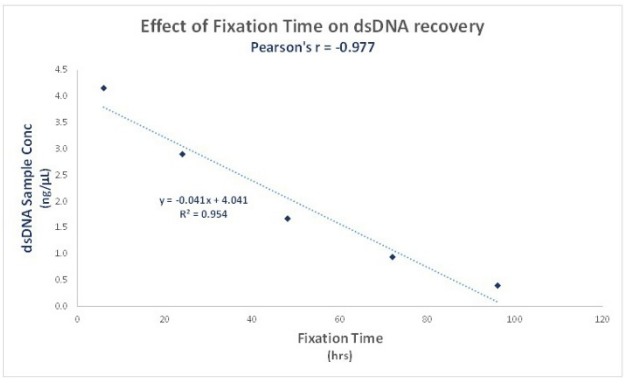
1990 The Effect of Prolonged Duration of Formalin Fixation on the Yield of Amplifiable DNA for Molecular Testing

Niru Nahar, Caleb King, Celeste N Powers, Gorge A Almenara, Catherine I Dumur, Michael O Idowu. Virginia Commonwealth University Health System, Richmond, VA.

Background: Formalin fixation (FF) is known to cause crosslinks between chromatin histones and the DNA helix leading to fragmentation of DNA and making extraction of amplifiable DNA more difficult. Standardization of the duration of FF and cold ischemic time (CIT) are now required for evaluation of breast cancer for *ERBB2* (*HER2*) by fluorescent in situ hybridization (FISH). There is currently no standardization of FF and CIT for nucleic acid extraction used for other molecular testing. The purpose of the study is to identify and standardize the optimal FF time and CIT for molecular testing, especially for small specimens or biopsies in our laboratory.

Design: Sections of normal colon from resection were divided into five equivalent pieces (2.0 mm each), all of which had a cold ischemic time of 1 hour (based on the current recommendation for *ERBB2* by FISH). The duration of formalin fixation was varied for each piece as follows: 6, 24, 48, 72 and 96 hours. The samples were processed using routine processor (Tissue- Tek VIP, Sakura). One H&E and 8 unstained 10 micron slides per tissue sample were used for DNA isolation using the EZ1 DNA Tissue Kit and the EZ1 Advanced XL DNA Paraffin Section Card, using a fixed elution volume of 50 µL. Amplifiable double stranded DNA (dsDNA) yield was estimated for each sample by measuring the dsDNA concentration obtained with the Qubit™ 2.0 fluorometer.

Results: There is a significant inverse correlation between the dsDNA concentration and fixation time (Pearson's r = -0.977, p = 0.0042), where the dsDNA concentration progressively decreased from over 4.0 ng/ul (6 hour fixation) to 1.7 ng/ul (48 hours of fixation) and lastly to 0.4 ng/ul (96 hour fixation), representing a reduction of over 10-fold in dsDNA yield.



Conclusions: Prolonged FF significantly reduces dsDNA yield. In our laboratory, while the dsDNA yield at 6 hours FF is optimal, we determined that 24 hour fixation is very good and practical for our workflow. Future studies to evaluate the impact of cold ischemic time are ongoing. Validation and standardization of the FF and CIT should ideally be performed byeach laboratory

1991 Switching from Performing Helicobacter Pylori Ancillary Studies "Upfront" to "On Demand": Impact on Reimbursements and Work Relative Value Units

Reeba Omman, Razvan Lapadat, Yi Zhou, Mohammed Atieh, Xianzhong Ding, Stefan E Pambuccian, Swati Mehrotra. Loyola University Medical Center, Maywood, IL.

Background: Detection of Helicobacter pylori (HP) is an essential component of gastric biopsy reporting. Ancillary studies (histochemical stains or immunostains) and laboratory protocols for their use ("upfront" routine use or "on demand" use after examination of the H&E-stained sections) in the detection of HP in gastric biopsies vary between laboratories. After the publication of the Rodger C. Haggitt Gastrointestinal Pathology Society (RCHGPS) recommendations, our laboratory switched from routine upfront use of Giemsa stains to the "on demand" use of immunostains. The aim of this study was to determine the impact of this change on the reimbursement and work relative value units (wRVU) in an academic institution.

Design: We identified all gastric biopsies from 9/22/2010-9/21/2015 in our database and determined the use of HP ancillary tests (Giemsa or HP immunostain). The data is divided into three periods: period 1 with upfront Giemsa stains (9/2010 to 4/2013); period 2 (4/2013-4/2014) with upfront Giemsa and on-demand IHC; and period 3 (4/2014-9/2015) on-demand IHC and/or Giemsa. Using the published reimubursement amount and wRVU value for Giemsa stain and immunohistochemistry from The Centers for Medicare and Medicaid Services (CMS) website we calculated the total and per case reimbursement and wRVU.

Results: We identified a total of 13,375 gastric biopsies in the study.

	Period 1	Period 2	Period 3
Total # biopsies	6147	3009	4219
# Giemsa stains	5655	2567	19
Giemsa $$	563,876.18	249,048.38	1897.48
Giemsa wRVU	3053.7	1385.64	10.8
# IHC stains	0	737	1667
IHC $$	0	79,075.20	149,099.96
IHC wRVU	0	573.7	1081.2
#Giemsa + IHC stains	0	597	5
Giemsa + IHC $$	563,876.18	328,123.58	150,997.44
Giemsa + IHC wRVU	3053.7	1959.34	1092.00
Per case $$	91.73	109.05	35.79
Per case wRVU	0.50	0.65	0.26

Conclusions: The implementation of the RCHGPS recommendations resulted in a threefold decrease in reimbursement ($91.73 to $109.05 to $35.79) and a two-fold decrease in wRVU (0.50 to 0.26) per gastric biopsy. These data show that more appropriate management of resources can lead to significant savings of health care dollars. This may seem counterintuitive in the present environment of fee for service, however improved test utilization may be of benefit for the evolving value based reimbursement model. At the present time this decline in wRVU and reimbursement should be kept in mind when determining wRVU targets, staffing levels and compensation of pathologists.

1992 Diagnostic Value of Flow Cytometry as a Screening Tool for Cerebrospinal Fluids in Patients with or without Prior Hematologic Malignancy

Nupam Patel, Karthik A Ganapathi, Stefanie Forest, Matthew B Thomsen, George Vlad, Govind Bhagat, Bachir Alobeid, Daniela Hoehn. Columbia University Medical Center, New York, NY.

Background: In patients with hematologic malignancies (HM), cerebrospinal fluid (CSF) involvement is invariably associated with worse prognosis. CSF Flow cytometry (FC) is vital for the diagnosis of HM and highly sensitive in identifying minimal disease

involvement. Recent studies suggest limited diagnostic utility in patients without HM history (HMhx). In this study we evaluate the diagnostic value of CSF FC, while also assessing its suitability as a screening tool in patients with or without HMhx.
Design: We reviewed CSF FC samples from the last 3 yrs., clinical history, cytomorphology (CM) and PCR results. Results were designated as positive, negative or indeterminate based on final diagnosis.
Results: 1000 cases were reviewed, 547 (55%) with HMhx and 453 (45%) without. M:F ratio was 1.2, median age was 58 yrs. (range: 1-89). Average number of antibodies used was 12 (range: 3-100). PCR and CM results were available in 100 and 94 cases, respectively. FC identified 78 positive cases, 73/547 with HMhx (13%, 7.3% of total) and 5/453 without (1.1%, 0.5% of total). 726 cases were negative, 380/547 with HMhx (70%; 38%) and 346/453 without (76%; 34.6%). 196 cases were indeterminate, 94/547 with HMHx (17%; 9.4%) and 102/453 without (23%;10.2%) of which 125 (12.5%) had low cellularity/viability. PCR was performed in 48/196 indeterminate cases, 33/94 with HMHx and 15/102 without and was positive in 9, all with HMHx. Prior CM review triggered submission of 94 cases (64 with, 30 without HMHx). 9 were positive (8/64, 9.5% with HMhx, 1/30, 1% without), 72 (76.5%) were negative, 13 (13%) inconclusive.
Conclusions: Our results show the diagnostic utility of CSF FC in pts. with HMhx while its value in pts. without HM remains limited, not warranting its incorporation as a screening test. Given the current climate of ascending healthcare costs, it is important to establish diagnostic tools that are cost effective and clinically useful.

Figure 1: Distribution of FSC FC Diagnoses in Relation to Prior HMhx

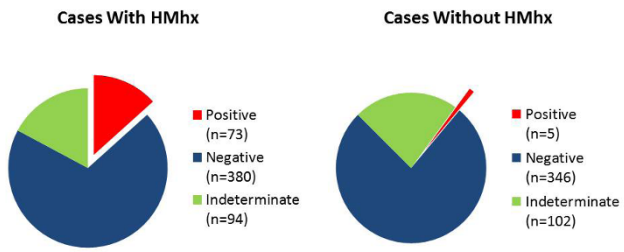


Figure 1: Positive, negative, and indeterminate CSF FC results tabulated according to patient population.

Table 1: Positive CSF FC Results By Subtype and Clinical History

Clinical History	AML (n=59)	ALL (n=61)	PTCL-NOS (n=63)	DLBCL (n=101)	BL (n=26)	CLL (n=63)	FL (n=21)	LPL (n=4)	MCL (n=21)	BNHL (n=63)	No HM History (n=453)
CSF FC Positive (%, n)	22% (13/59)	8% (5/61)	35% (22/63)	2% (2/101)	4% (1/26)	16% (10/63)	19% (4/21)	50% (2/4)	43% (9/21)	2% (2/83)	1% (5/453)

Table 1: AML: acute myeloid leukemia, ALL: acute lymphoblastic leukemia/lymphoma, CLL: chronic lymphocytic leukemia/small lymphocytic lymphoma PTCL-NOS: Peripheral T-cell leukemia/lymphoma not otherwise specified, DLBCL: diffuse large B-cell lymphoma, BL: Burkitt lymphoma, FL: follicular lymphoma, LPL: lymphoplasmacytic lymphoma, MCL: mantle cell lymphoma, and BNHL: B-cell non-Hodgkin lymphoma.

1993 Diagnostic Utility and Concordance of Cytopathology and Flow Cytometry of Cerebrospinal Fluid in Lymphoblastic Leukemia/Lymphoma

Nupam Patel, Daniela Hoehn, Karthik A Ganapathi, Govind Bhagat, Bachir Alobeid. Columbia University Medical Center, New York, NY.

Background: The evaluation of cerebrospinal fluid (CSF) for lymphoblastic leukemia/lymphoma (ALL) patients is often rendered by a combination of diagnostic modalities, such as cytology and flow cytometry (FC). Prior studies have noted that FC is superior to cytopathologic examination in the diagnosis of hematologic malignancies. Hence, we evaluated the diagnostic utility and concordance of FC with CSF cytopathology in ALL.
Design: We searched our departmental database for the past 15 years to identify cases of ALL where concurrent CSF FC and cytopathologic examination was performed.
Results: A total of 174 ALL cases were initially reviewed (age range: <1 up to 87 years, 113 children and 61 adults; M:F ratio of 1.7:1) of which 52 patients (30%) [41 (79%) B-ALL and 11 (21%) T-ALL] had concurrent FC and cytopathologic examination of CSF specimens. A total of 131 CSF specimens from these patients were analyzed (specimen range: 1-10 per patient, average:2). The diagnoses were categorized as positive, negative or inconclusive.
Ninety four of 131 cases (72%) showed concordance between FC and cytopathology with 80 (85%) being negative by both FC and cytopathology, 13 of 94 (14%) positive by both, and 1 (1%) inconclusive by both modalities. Twenty four of 131 cases (18%) showed discrepant results with 8 of 24 (33%) showing a positive result by FC and negative (3) or inconclusive (5) by cytology. No positive cytology case was negative by FC.Twelve of 24 cases (50%) represented negative FC results with inconclusive cytopathology results and 4 of 24 cases (17%) represented inconclusive results by FC and negative cytopathology. Thirteen of 131 cases (10%) had insufficient material for FC due to low cellularity and 2 (15%) of these cases were suspicious for blasts by cytopathology.

Figure 1: Concurrent CSF Specimens Submitted for FC and Cytopathologic Analysis

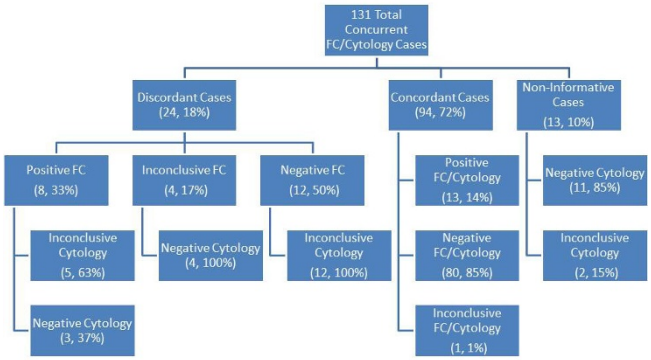


Figure 1: The distribution of concurrent CSF specimens based on FC and cytomorphology.

Conclusions: Our findings indicate that the diagnostic yield of FC is superior to cytomorphologic examination; however, given the inherent low cellularity of CSF samples, adjunct cytopathology should be routinely performed for maximal diagnostic yield. Discrepant cases could be further analyzed by molecular methods to assess CSF involvement by ALL.

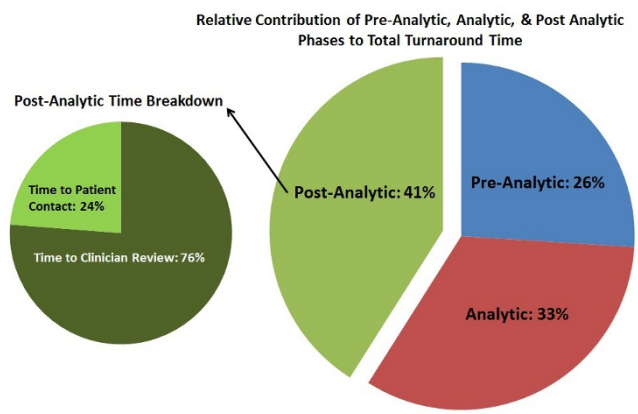
1994 Turnaround Time In Surgical Pathology: The Patient Perspective

Garrison Pease, Elisheva Shanes, Charnell Johnson, William Watkin. University of Chicago (NorthShore), Evanston, IL.

Background: Turnaround time (TAT) is a closely-tracked quality indicator. TAT is calculated as the interval between specimen accessioning and signout (analytic phase) and for routine cases, many labs achieve the CAP recommended TAT of 2 days. Patients experience a longer total TAT which includes pre-analytic and post-analytic testing phases. While pre-analytic variables such as courier delays are well known, post-analytic variables have not been carefully studied. It is assumed that the advent of computerized reporting, immediate delivery of pathology results to electronic medical records (EMR), and wide use of patient access portals (PAP) enables patients to receive their results expeditiously.
At our institution, surgical pathology results are transmitted to the EMR 1 hour after signout. Results are provided to patients by phone or release to a PAP either actively by the clinician after result review or automatically in 3 days without clinician review. In this study, we measure relative contribution of each testing phase to the total TAT and evaluate the effectiveness of computerized reporting in patient result delivery.
Design: 151 consectuive outpatient biopsies over a 3 week period were tracked. Time of specimen collection, accessioning, and signout were derived from PowerPath. Time of physician result review, time of result release to the PAP or time results were communicated to the patient were extracted from the EMR (Epic). The time interval for each step was calculated and the relative contribution of each testing phase to the total TAT was assessed.
Results: The average TAT for each component of the TAT is illustrated in Table 1.

Components of Total TAT	Total	Pre-Analytical	Analytical	Total Post-Analytical	Components of Post-Analytical	
					Clinician Review	Patient Contact
Average (days)	4.03	1.06	1.31	1.65	1.26	0.39
Proportion	1.0	0.26	0.33	0.41	0.76	0.24

The relative contribution of each component to the TAT is illustrated in Figure 1.



Conclusions: After careful assessment, the post-analytic phase consumes a remarkable 41% of total TAT, 76% of which is due to delay of clinician review after specimen signout. Despite rapid electronic report delivery at signout, results are often not expeditiously reviewed and communicated to patient.

1995 Single Versus Multiple Levels for Routine Endometrial Biopsies: Single Institution Experience

Anna Plotkin, Linda R Kapusta, Keiyan Sy, Blake Gilks. University of Toronto, Toronto, ON, Canada; University of British Columbia, Vancouver, BC, Canada.

Background: A number of step-sections (levels) are usually prepared for small endometrial biopsy specimens. The number of initial levels can vary arbitrarily from one to three in different pathology departments. At Trillium Health Partners, three levels are evaluated from each endometrial biopsy tissue block. The purpose of this study was to evaluate whether assessment of only the second level would be sufficient for diagnosis, and therefore first and third levels are not necessary.

Design: Endometrial biopsy and or curetting cases accessioned between January 1, 2015 and June 30, 2015 were retrieved from the Department of Anatomical Pathology at Trillium Health Partners Credit Valley Site. 320 cases with three levels were retrospectively reviewed. Level 2 of each of the 320 endometrial biopsy cases was reviewed to assess for adequacy of diagnostic material and necessity for reviewing levels 1 and 3.

Results: After reviewing level 2, the diagnosis was rendered in 317 cases (99.06%). Three cases (0.94%) required assessment of additional slides. One case showed absence of endometrial tissue on level 2 and a few atrophic glands were present on level 1. Another case showed endometrial stroma only on level 2 and a few atrophic glands on level 3. The third case showed few atypical glands on level 2 suggestive for atypical hyperplasia. Level 3 showed unequivocal atypical hyperplasia. This last case was the only case where the original pathologist requested additional levels (4-6).

Conclusions: Assessment of a single slide per block (originally designated as level 2) is sufficient for assessment of endometrial biopsies. Preparation of three levels is not necessary; after evaluation of a single slide additional level can be ordered if necessary. This approach will save time for both professional and technical staff and reduce costs of slide storage.

1996 Use of Barcode Tracking in Routine Histology Processing Significantly Decreases Pre-Analytical Errors

Mahboubeh Rahmani, Kristi Bedrossian, Elizabeth Genega. Tufts Medical Center, Tufts University School of Medicine, Boston, MA.

Background: Given the manual nature of processing, surgical pathology case components including requisitions, specimen containers, cassettes and slides are susceptible to mislabeling. Continuous monitoring of the rate of mislabeled cases is useful to detect the causes of such mislabeling, understand the consequences of mislabeled specimens, implement safer practice, and help to fulfill the Joint Commission National Patient Safety Goal to identify patients and specimens correctly. We monitored mislabeled components of cases pre and post implementation of Cerner CoPath Advanced Barcode and Tracking (AB&T).

Design: Mislabeled case components were reported by technical and professional staff using a confidential QA form beginning 01/2015. Follow up and correction of the errors were documented and the cause for each error was tracked. During the third quarter of 2015, AB&T was implemented at all aspects of specimen processing with emphasis on grossing and histologic processing. Technical and professional staffs were educated on the proper use of the system.

Results: Table 1 shows the number/percentage of mislabeled blocks and slides. The percentage of mislabeled slides in the third quarter in comparison to the first and second quarters was decreased 12 and 10 times, respectively (from 0.7% in the first quarter and 0.6% in the second quarter to 0.06% in the third quarter). The first and second quarters had 0.19% and 0.07% mislabeled blocks respectively; the percentage of mislabeled blocks in the third quarter was the same as the second quarter after the initial phase of AB&T implementation. The third quarter showed a significant decrease in mislabeled slides. The origin of most block labeling errors are either pre-accessioning (in clinic) or at accessioning, both of which are not included in the scope of the AB&T system.

Time Period	Total number of blocks	Number of mislabeled blocks(%)	Total number of slides	Number of mislabeled slides(%)
January- March 2015	14781	28(0.19)	36576	256(0.70)
April- June 2015	17170	12(0.07)	41821	257(0.61)
July- September 2015	16803	12(0.07)	44022	26(0.06)

Conclusions: Proper application of a barcode tracking system like AB&T at the various stages of routine processing of surgical pathology specimens significantly enhanced accuracy of patient identification by decreasing labeling errors. Our study confirms the impact of applying barcode tracking systems in decreasing pre-analytical errors and improving patient care.

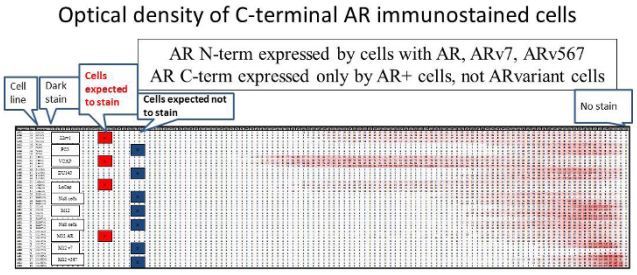
1997 Quantifying Protein Levels in Cells by Violin Plots: A Facile, Readily Visualized Method

Nicholas P Reder, Jonathan C Henriksen, Lawrence True. University of Washington, Seattle, WA.

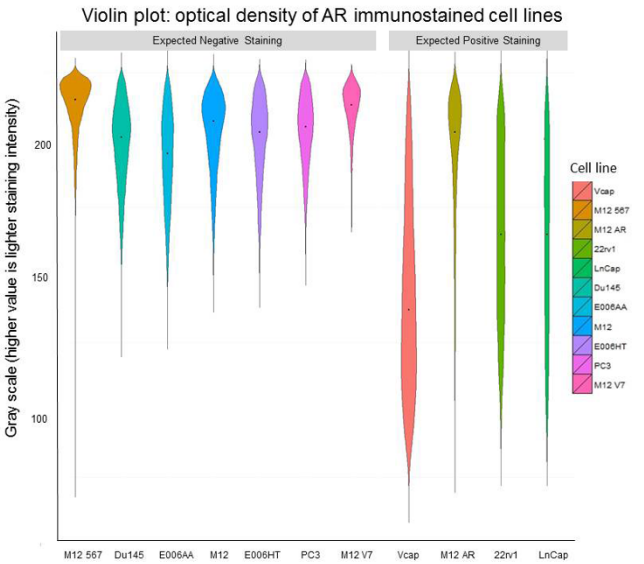
Background: The intensity of immunohistochemical stains of antigens in tumor cells is a metric for protein levels. Expression levels of proteins such as estrogen receptor and her2 guide therapy. By convention immunostains are visually scored. However, visual interpretation of immunohistologic stains is subject to observer variability. We developed data visualization and analysis methods that assess immunostain intensity independent of the pathologist's visual assessment.

Design: Sections of tissue microarrays of cell lines expressing different levels of androgen receptor (AR) full length, AR variant 7, and AR variant 567 were stained with antibodies to C-terminal AR (Spring SP 242; absent in AR variants). Slides were digitized on a whole slide scanner. Using a Nuclear Algorithm, nuclei were segmented using thresholds. The number of nuclei at each gray level (0 - 255) in the DAB color channel were then tabulated for each cell line quadruplicate.

Results: The average maximum nuclear gray level (intensity of stain) correlated with AR and AR variant values determined by PCR transcript assays. C-terminal AR is expressed only by cells expressing AR full length (LnCAP, 22rv1, VCAP, M12AR), not by AR variants. These results can be readily seen in Figure 1.



Quadruplicates are concordant. Distribution of stained cells is indicated in a violin plot, which shows median intensity and the number of nuclei at each gray level for each cell line. ANOVA tests with Tukey multiple comparisons tests detected relatively weak M12AR staining, suggesting that the AR full length gene transfected only a minority of M12 cells. This statistical finding can be easily confirmed by visual inspection of a violin plot.



Conclusions: Violin plots are a facile method of displaying and quantifying the intensity of immunostains, avoiding the "black box" nature of statistical tests. This semi-quantitative method is not subject to the variability of visual interpretation of immunohistologic stains.

1998 Does Expert Review of Testis Biopsies for Infertility Improve Clinicopathologic Correlation?

Brian D Robinson, Aaron Bernie, Peyman Tavassoli, Theresa Scognamiglio, Francesca Khani, Peter N Schlegel. Weill Cornell Medical College, New York, NY.

Background: Pathologic findings in testis biopsies correlate with sperm retrieval rates (SRR) during microdissection testicular sperm extraction (mTESE). Thus, accurate histologic diagnosis is important for patient management, particularly in men with unsuccessful mTESE, as biopsy results may determine whether subsequent mTESEs are attempted. This study assessed accuracy of testis biopsy interpretation and identified pitfalls leading to incorrect histologic classification.

Design: 70 testis biopsies from mTESE originally reported by general surgical pathologists as maturation arrest (MA) (38 early and 32 late) were reviewed by a single urologic pathologist blinded to SRR. Biopsies were re-classified as either Sertoli cell only (SCO), early MA, late MA, or hypospermatogenesis. Correlation of original and reclassified diagnoses with SRR was assessed. Potential reasons for misclassification were sought in discrepant cases.

Results: Diagnoses were concordant in 50 (71%) biopsies [35/38 (92%) early MA and 15/32 (47%) late MA]. All 3 discrepant early MA were reclassified as SCO. These 3 SCO cases had tubules with immature Sertoli cells and/or Sertoli cells with round basally-located nuclei mimicking germ cells. 8 late MA were reclassified as early MA and 9 as hypospermatogenesis. 6 reclassified late MA cases had tubules with sloughed cells with pyknotic nuclei mimicking spermatids, and 1 case had poorly preserved primary spermatocytes mistaken for spermatids. 1 late MA case had both

sloughed cells and poorly preserved primary spermatocytes. All 9 late MA reclassified as hypospermatogenesis demonstrated only 1-2 late spermatids/spermatozoa in rare tubules that likely were overlooked.
SRR was 53% (20/38) in original early MA cases and 59% (19/32) in original late MA cases. After review, SRR dropped to 47% (20/43) in early MA cases and rose to 67% (10/15) in late MA cases. Only 1 (33%) SCO case had successful sperm retrieval while SRR was 89% (8/9) in hypospermatogenesis cases.
Conclusions: Only 71% of biopsies were properly classified at original diagnosis. Pathologists must be aware of potential pitfalls when interpreting infertility biopsies, including misinterpretation of immature Sertoli cells as germ cells as well as sloughed tubular cells and poorly preserved primary spermatocytes as spermatids. A diligent search for rare late spermatids/spermatozoa is imperative as these men have the highest SRR. Given their complexity, rarity, and potential pitfalls, expert review of infertility biopsies by urologic pathologists can improve clinicopathologic correlation and allow for optimal patient management.

1999 Identifying Targets for Reducing Immunohistochemical Utilization: An Audit of 18,760 Cases

Lisa M Rooper, Jonathan I Epstein, Ashley Cimino-Mathews. Johns Hopkins Hospital, Baltimore, MD.
Background: While immunohistochemistry (IHC) can provide a wealth of diagnostic and prognostic information, excessive use of IHC significantly increases the cost of pathologic examination. Moreover, escalating regulation of IHC billing often forces pathology departments to absorb the expense of surplus stains. Although countless studies have compared the utility of specific staining panels, there is a paucity of research evaluating more global patterns of IHC usage in routine practice. We retrospectively reviewed all IHC performed over 3 years to identify targets for reducing IHC utilization.
Design: We identified all surgical pathology cases that underwent IHC at a large academic medical center from July 2012 to June 2015; outside consultation cases were excluded. All stains performed were tabulated. Specimens were categorized by organ system, and pathologists were classified by rank (senior indicating >10 years in practice) and expertise (experts having subspecialty fellowship or consultation experience). We compared case proportions stained using the Fisher exact test and mean stains with a two-tailed t-test.
Results: Overall, 70,121 stains were ordered on 18,760 cases (mean 3.7, median 3.0). While just 3,620 cases (19.3%) had >5 stains, they accounted for 33,288 of stains performed (47.5%). Prognostic and staging protocols alone were responsible for 11,256 stains (16.1%). Across all organ systems, breast and hematolymphoid cases had the highest rate of staining and most mean stains performed. Junior pathologists performed IHC on a higher proportion of their total caseload than senior pathologists (15.0% vs. 12.4%, p<0.001), accounting for an estimated 8,028 excess stains. Non-experts ordered more mean stains per case than experts (4.29 vs. 3.36, P<0.001), producing 10,410 extra stains. A variety of redundant stains were identified between and within cases.
Conclusions: Our study of IHC practice patterns highlights several targets for reducing immunostain utilization. A relatively small proportion of cases account for almost half of IHC performed; limiting redundant and excessive staining in only a subset of such cases could significantly lower usage. Differences in IHC ordering across pathologist rank and expertise suggests that intradepartmental consultation also might forestall many excess stains. Additionally, while prognostic and staging protocols are often unavoidable, more judicious application could limit a sizeable component of IHC use. Attempts to decrease IHC in the academic setting must account for its separate educational and investigational value.

2000 Switching from FDA-Approved to Laboratory-Developed HER2 IHC Testing Decreases Costs by 85%: A Micro-Costing Analysis

Carolyn Rysgaard, Ellen Abusada, Lisa Horning, Rose Meyer, Kent Becker, Andrew M Bellizzi. University of Iowa Hospitals and Clinics, Iowa City, IA.
Background: HER2 testing, using immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH), is standard of care in invasive breast and advanced esophageal/gastric cancer. Laboratories use FDA-approved kits or laboratory-developed tests (LDTs). HER2 IHC is scored semi-quantitatively, 0-1+ (negative), 2+ (equivocal), 3+ (positive), with 2+ cases typically reflexed to FISH. Driven principally by reagent shortages but also by perception of possible cost savings, we recently validated a HER2 LDT using the rabbit monoclonal antibody SP3 as a replacement for the FDA-approved Dako HercepTest. We selected SP3 based on data that it reduces the rate of 2+ IHC without sacrificing sensitivity. Herein, we present a micro-costing analysis of our FDA-approved vs. LDT-IHC-based HER2 testing programs.
Design: To determine the cost per HER2 test we obtained: costs of FDA-approved kits/primary antibody, detection chemistry, and labor (based on in-house time motion study) and antibody dilutions (to determine per slide primary antibody cost); we also ascertained Medicare reimbursement rates. To determine impact of the switch on 2+ IHC, we searched the AP information system for all HER2 IHC orders over a 3-year period (25 months pre- and 11 months post-intervention), limiting the analysis to carcinomas of the breast/upper GI tract.
Results: Following the switch, HER2 IHC costs decreased from $231 to $18.01 per slide, a 92% savings (Table 1). We analyzed data from 556 HER2 IHC cases (380 primary breast, 78 primary upper GI, 82 metastatic breast, 16 metastatic GI), 412 stained with HercepTest and 144 with SP3. 2+ IHC results decreased from 23% to 10% (Table 2), while reflex-FISH positives increased from 8% with HercepTest to 25% with SP3.

	HercepTest	SP3	HER2 FISH
Micro-Cost	Primary antibody: $214.29 Detection chemistry: $8.40 Labor: $8.31	Primary antibody: $1.70 Detection chemistry: $8.00 Labor: $8.31	Supplies: $235.02 Labor: $23.77
Total Cost	$231.00	$18.01	$258.79
Medicare Reimbursement	$180.00	$180.00	$180.00
Reimbursement Less Cost	-$51.00	+$161.99	-$78.79

	Negative (0-1+)	Equivocal (2+)	Positive (3+)
HercepTest (n=412)	267(65%)	93(23%)	52(13%)
SP3 (n=144)	114(79%)	14(10%)	16(11%)

Conclusions: A carefully validated HER2 IHC LDT offers dramatic cost savings over an FDA-approved kit, without sacrificing quality. Given 230K newly diagnosed US breast cancers each year and market saturation by HER2 FDA-approved kits, switching to LDTs (saving ~$200 per test) would save US healthcare tens of millions of dollars.

2001 Accuracy of Intraoperative Consultation Rendered by General Pathologists in a Scenario Where Well-Defined Decision Algorithm Is Followed

Sherine Salama, James Richter, Tanya Pulver, Boris Winterhoff, Mahmoud Khalifa. University of Toronto, Toronto, ON, Canada; University of Minnesota, Minneapolis, MN.
Background: Over the past decade, most North American academic pathology laboratories have moved toward some degree of subspecialization driving management decision algorithms with a higher degree of precision. Intraoperative consultation (IOC) remains an area of general practice even within subspecialized departments. This study investigates the accuracy of IOCs rendered in a general pathology setting where surgeons integrate these results in a well-defined algorithm, developed with the input of specialized pathologists.
Design: Surgical decisions to perform staging lymphadenectomy in patients with endometrial adenocarcinoma in our institution were determined according to a well-defined algorithm on the basis of the IOC assessment of tumor size, grade and depth of invasion in the hysterectomy specimen. We analyzed cases between 1/2003 – 6/2015 where tumor size, depth of invasion, and grade were recorded from the Frozen Section (FS) as well as the permanent sections. Surgical and clinical patient outcome were obtained.
Results: FS were examined in 801 cases. Surgeries were performed by 18 surgeons; IOCs were conducted by 16 general pathologists. Of the 801 cases, on FS 381 had documented depth of invasion (58 had no tumor and 362 cases had no documented depth). FIGO grade was recorded in 354 cases (64 had no tumor and in 383 cases no grade documented). The FS depth of invasion had an overall accuracy of 0.92 (95% CI: 0.89-0.94) and deep invasion had a false negative rate of 0.08 (95% CI: 0.05-0.11). Deep invasion was under-called in 25 cases and, as a result, 5 of these patients did not have staging lymphadenectomy. None of these patients had tumor recurrence. The FS FIGO grade had an overall accuracy of 0.83 (95% CI: 0.79-0.87) and high grades (FIGO 2 or 3) had a false negative rate of 0.21 (95% CI: 0.16-0.26). High FIGO grades were under-called in 55 patients and, as a result, 21 patients did not have staging lymphadenectomy. Only one of these 21 patients had lung recurrences at 4 and 8 years postoperatively.
Conclusions: This audit suggests that an IOC service provided by general pathologists is a safe practice in this example where well-defined decision matrix is followed. More communication regarding the value of consistently reporting and accurately assessing critical parameters that feed into this algorithm is needed, to enhance the model and reduce false negative rates.

2002 Elimination of Cutting and Saving Unstained Sections of GI Biopsy Specimens Yields Significant Benefits with No Untoward Effects on Quality

Safia N Salaria, Craig W Self, Mary F Abbuhl, Omar Hameed, Lan L Gellert. Vanderbilt University School of Medicine, Nashville, TN.
Background: At our tertiary academic medical center, gastrointestinal (GI) biopsies constitute 31% of total 61,428 surgical pathology specimens and 58% of biopsies accessioned yearly. Historically, the histology sectioning protocol of such biopsies involved the prospective production of unstained slides as intervening levels between H&E sections, of which <10% were being utilized. In an effort to improve utilization of laboratory resources, this practice was discontinued based on consensus among GI pathologists that it would be unlikely to impact patient care in a negative manner. The goal of this study was to retrospectively evaluate the financial impact of this protocol change and its effect on quality.
Design: Total number of slides cut, unstained section utilization rates and turnaround times (TAT) for GI biopsies were compared 6 months before and 6 months after implementation of the protocol change. The material and labor costs were calculated, and both pathologists and histotechnologists were also surveyed to further determine the impact of the protocol change.
Results: A total of 271,262 slides were generated by the histology lab prior to implementation, including 36,319 (12.3%) unstained interval sections, versus 221,261 and, 16,939 (7.7%) after implementation, respectively (P<.0001). Unstained section utilization rates were 14.6% before, vs. 20.9% after implementation (P<.0001). There was with no significant change in adult GI biopsy TAT.

The estimated annualized monetary savings associated with the almost 20K reduction in unstained sections included $7000 in material costs and $100,000 in labor costs (based on 2.4 FTEs).
88% of the pathologists surveyed had no negative feedback in reference to both the quality of slides & the overall TAT , including complex cases that require ancillary studies.
100% of histotechnologists surveyed reported a significant increase in job satisfaction and significantly positive impact on workflow, including an approximately 40% reduction in total time e.g. leveling paraffin blocks, cutting and labeling unstained sections,due to the protocol change.
Conclusions: Elimination of cutting and saving unstained sections of GI biopsy specimens results in significantly better utilization of lab resources and increased histotechnologist job satisfaction, whilst maintaining TAT and quality of patient care. This is especially important given the changing healthcare environment.

2003 Pre-Analytical Factors Involved in the Clinical Analysis of a Comprehensive Next-Generation Sequencing Panel

Alaa A Salim, Rajyalakshmi Luthra, Rajesh Singh, Keyur P Patel, Mark Routbort, Bedia A Barkoh, Jawad Manekia, Sinchita Roy Chowdhuri, Russell Broaddus, Hui Chen. MD Anderson Cancer Center, Houston, TX.
Background: Screening for somatic mutations is an integral part of the comprehensive work up for cancer patients. These mutations can be important in predicting disease prognosis and guiding of personalized cancer therapy. The advent of massively parallel next-generation sequencing (NGS) allows for screening large number of genes. Tumor sample factors are critical for generating accurate results to guide therapeutic intervention. We evaluated pre-analytical factors that affect the performance and success of comprehensive NGS analysis of solid tumors from formalin fixed and paraffin embedded tissues (FFPE) and cytology specimens.
Design: 267 consecutive solid tumors (FFPE and/or cytology) were tested for genetic aberrations in 409 full length genes by NGS using Ion Proton and IonAmpliSeq Comprehensive Cancer panel (Life Technologies). We evaluated the success rate of sequencing cytological and surgical specimens, by comparing tumor size, biopsy procedure, tumor type, and tissue processing, such as decalcification and block age, to determine the factors affecting the outcome of NGS.
Results: The overall success rate of NGS was 96%; the remaining 4% failed either at the DNA quantity or library preparation steps. DNA concentration had a great impact on NGS success with 99.6% (231/232) success with DNA concentration $\geq$ 2 ng/ul; 75% (9/12) success with 1.5-2 ng/ul; and 8% (1/12) success with concentration $\leq$ 1.5 ng/ul. Surgical specimens with large circled tumor ($\geq$ 60mm^2) have a higher success rate (107/107;100%) than that of smaller circled tumor (<10 mm^2) (29/38; 76%), and cytology smears (6/8; 75%). Decalcified specimens processed with formic acid have 100% success rate (3/3); however block age did not a have significant effect on NGS success.
Conclusions: In this study, we show that certain pre-analytical factors such as procedure type and circled tumor size should be taken into consideration when selecting tissues for comprehensive NGS panel using Ion Proton. These factors contributed to DNA yield and ultimately affect the final sequencing success.

2004 Tumor Area Measurement as a Parameter for Determination of Specimen Adequacy in the Selection of EBUS-FNA Specimens for Mutational Analysis by Targeted Next Generation Sequencing (NGS)

Sadia Sayeed, Laura M Warmke, Michael O Idowu, Celeste N Powers, Adele O Kraft. Virginia Commonwealth University Medical Center, Richmond, VA.
Background: EBUS-FNA (endobronchial-ultrasound with fine needle aspiration) permits sampling of lung masses and mediastinal lymph nodes for diagnosis and staging of lung cancer patients presenting with unresectable advanced disease. The cells and/or tissue acquired in this procedure may often be the only available source for molecular testing, which is essential for tyrosine kinase inhibitor (TKI) therapy selection. Minimum proportion and number of cancer cells needed for mutational analysis by NGS have been used for assay validation. Measurement of the area occupied by the malignant cells on cell block slides was performed to determine its utility for evaluating specimen adequacy.
Design: Using the electronic medical record (Cerner) database, 20 cases of EBUS-FNA material that underwent a 50-gene panel targeted NGS testing were identified between April 1, 2014 to August, 30 2015. The H&E slide on file was reviewed. Measurement of the total tumor area as the sum of small tumor fragments was performed utilizing a Nikon Eclipse Ni-U microscope with APO objectives and attached Nikon DS-Fi2 Color Camera 5mp with NIS-Elements D 4.10.01 software. The percentage of tumor cells in relation to the total number of nucleated cells was visually estimated as well as the percentage of necrotic material present on the slide by two independent screeners.
Results: All 20 cases had the histological sections entirely processed for DNA extraction with no microdissection. The tumor area was >0.20mm^2 in all cases. The percentage of tumor in relation to total number of nucleated cells was >25% overall, with a majority of cases (15/20) ranging 60-100%. All cases had less than 20% necrotic material, 19/20 had necrosis of 0-10%. 8 of 20 cases had pathogenic variants in the following genes: EGFR, BRAF, and KRAS. In the remaining cases, NGS was successfully performed but no pathogenic variant was identified.
Conclusions: A tumor area of >0.20 mm^2 in conjunction with >25% tumor cells and <20% of necrotic material can be used as positive selection parameters for specimens requiring molecular testing using targeted NGS in cell block material obtained by EBUS-FNA without need for any additional dissection procedure.

2005 Prospective Quality Assurance of Breast Core Biopsies Minimizes Errors and Improves Patient Safety

Sejal S Shah, Natalie S Campbell, Gary L Keeney, Beiyun Chen, Daniel W Visscher. Mayo Clinic, Rochester, MN.
Background: Quality assurance (QA) procedures are not well developed to evaluate the diagnostic accuracy of breast core needle biopsies (CNB). The aim of this study was to assess the inter-observer variability and disagreement rate for breast CNB among pathologists.
Design: All breast CNBs, during a fifteen month period, that were not seen by at least 2 breast pathologists were blinded and reviewed for QA by a breast pathologist. QA was performed before the case was signed out and within 24 hours of initial review. Disagreements were classified as major or minor as determined by the impact on patient management. Opinions on cases of major disagreement were obtained from additional breast pathologists by blinded review followed by case discussion. Clinical and surgical follow up was recorded for all cases.
Results: Of the total 2236 breast CNB, 1475 cases (66%) were submitted for QA. After initial QA and consensus review, 1403 cases (95%) were in agreement with the primary pathologist, 15 cases (1%) had minor disagreement and 57 cases (4%) had major disagreement.

Table 1: Major Disagreement Cases after Initial and Consensus QA review

Change in diagnosis after QA and consensus review (n)	Surgical follow up (n)
Benign to ALH (6)	No excision (6)
Benign to ADP (8)	Benign (4), ALH (1) *ADH (1), no excision (2)
Benign to FEA (7)	Benign (3), FEA (2), *ADH (1), no excision (1)
Benign to apocrine atypia (2)	Benign (1), *DCIS (1)
Benign to ADH (12)	Benign (3), ALH (3), *ADH (1), *DCIS (3), no excision (2)
Atypical to benign (8)	*No exicision (8)
ADH to DCIS (1)	*DICS (1)
DCIS to ADH (3)	*ADH (1), DCIS (1), no FU (1)
DCIS to invasive (1)	*Invasive (1)
Benign to DCIS (1)	*DCIS (1)
Ductal to lobular atypia (3)	Benign (1), no excision (2)
Lobular to ductal atypia (3)	Benign (1), *DCIS (1), no excision (1)
FEL to FA (1)	*No excision (1)
FA to FEL (1)	FA (1)

*: Significant impact on patient management.
ADP: Atypical ductal proliferation defined as either cytological or architectural atypia, FA: Fibroadenoma, FEL: Fibroepithelial lesion, FU: Follow up
Conclusions: Majority of the cases (75%) with inter-observer variability and major disagreement were related to the presence or absence of atypia. In our analysis 37% of the major disagreement cases and 1.4% of total cases submitted for QA had significant impact on patient management. This is the first study to measure clinical significance of diagnostic discordance in breast CNB in a real time, clinical practice setting.

2006 Histologic Evaluation of the Effects of Tissue Decalcification on the Immunohistochemical Markers in Bone Marrow Specimen

Marianna Shvartsbeyn, Mohamed E Salama, Rajan Dewar. New York University, New York, NY; University of Utah, Salt Lake City, UT; University of Michigan, Ann Arbor, MI.
Background: The performance characteristics of each assay performed in the clinical immunohistochemistry laboratory must be appropriately validated before it is applied for clinical use. Recently published expert consensus recommendation on the decalcified material is to test a selected set of commonly ordered markers and to correlate the results with the routinely processed (control) tissues and ancillary test results, if available. We sought to evaluate the effect of decalcification on the immunoperoxidase expression in the tonsillar tissue, which has not been previously studied. We utilized digital imaging to quantify the staining intensity for each of the marker studied on the tonsillar tissue. By extension, we tested this approach as a practical method to validate immunostains in BM biopsy specimens.
Design: A select panel of eleven IHC markers most commonly utilized for evaluation of bone marrow core biopsies was studied on a decalcified tonsillar tissue and was tested in parallel with the routinely processed section. Several bone marrow core biopsies were retrieved from the archives and reviewed retrospectively for comparative analysis.
Results: We observed that different antigens showed different sensitivity to the same decalcification regimen; it is plausible that antigenic epitopes have different vulnerability to different decalcifying agents. The intensity of staining was significantly decreased in eight out of eleven immunoperoxidase stains evaluated. When the staining intensity was compared between the decalcified section and the tissue control in bone marrow specimens, Ki-67 was markedly decreased, CD34 and CD117 did not show any appreciable change in the staining quality or intensity, and all other immnomarkers were affected to various degrees. By objective image analysis performed on tonsil only, CD10, CD34 and MPO were not significantly affected.
Conclusions: Decalcification may substantially alter the intensity of the IHC stains. This study exemplifies a practical and useful approach that could be employed to validate the immunostains in demineralized tissue for accreditation purposes, quality assurance and quality improvement.

2007 Napsin Expression in Metastatic Colorectal Carcinoma to the Lung, a Potential Pitfall in the Immunohistochemical Assessment of Adenocarcinomas in the Lung, Identified In Immunohistochemistry Validation

Manmeet Singh, Michael Clarke, Ashley S Freyre, Shiraz S Fidai, Melody Lee, David Stephen, Elizabeth Wiley, John V Groth. University of Illinois Hospital & Health Sciences System, Chicago, IL.

Background: Napsin A is routinely used as a marker for the diagnosis of primary lung adenocarcinoma. Since its introduction studies have identified Napsin A expression to be common in other tumors, such as clear cell ovarian carcinoma. Immunohistochemistry validation is required before daily use. We discovered abberant Napsin A expression, during its validation, utilizing Tissue microarrays (TMAs) to assess Napsin A expression in metastatic tumors to the lung, particularly with colorectal adenocarcinomas. We therefore aim to describe the unique findings of our validation.

Design: We identified 93 total cases of primary (75) and secondary (19) lung malignancies, using a retrospective search of cases at The University of Illinois Hospital & Health Sciences System (2007-2013). Two TMAs were constructed. Napsin A IHC was performed using 5-mm sections and a Ventana Benchmark XT autostainer for Napsin A (Cell Marque) and scored as positive if expression was intense or weak/moderate and present in>25% of cells.

Results: Napsin A expression was identified in 83% of lung primary adenocarcinomas (33/38 nonmucinous and 0/2 mucinous adenocarcinomas), 0% of squamous cell carcinoma 0/25, 50% of adenosquamous carcinoma 1/2, 33% of large cell carcinoma 1/3, carcinoid 0/1, large cell neuroendocrine 0/1, small cell carcinoma 0/2 and 0% of synovial sarcoma 0/1. Napsin expression was identified in 16% of metastases to the lung with 100% of colorectal adenocarcinomas with an apical granular staining 3/3, and 0% of the remaining (leiomyosarcoma 0/7, papillary thyroid carcinoma 0/1, melanoma 0/1, germ cell tumor 0/1, enodometrioid carcinoma 0/1, esophageal adenocarcinoma 0/1, paraganglioma 0/1, osteosarcoma 0/1, renal cell carcinoma, clear cell type 0/1, thyroid Hurthle cell carcinoma).

Conclusions: TMAs allow for a wide variety of tissue types to be tested, and as a result allows for a greater understanding of protein expression in a variety of tumors, which allow them to be easily used for immunohistochemistry validation. Napsin A is useful for identifying tumors of nonmucinous bronchogenic differentiation; however, pitfalls exist. This study highlights that discoveries can be made while undergoing immunohistochemistry validation and that when dealing with mucinous tumors that metastatic colorectal adenocarinoma should be considered when napsin A expression is identified, particularly with apical granular staining.

2008 Pathologists Discussing Diagnoses with Patients: A Pilot Study in Lymphoma Clinic

Lauren B Smith, Brian Tolle, William Sherman, Scott R Owens, Mark Kaminski. University of Michigan, Ann Arbor, MI.

Background: Traditionally, pathologists review diagnostic material and the hematologist/oncologist (H/O) conveys the diagnosis to the patient. Issues may arise as the H/O may not be familiar with the diagnostic process, including the quality of the material, the utility of ancillary studies, and/or any diagnostic difficulty or uncertainty that may exist. We hypothesized that it may add value to have a hematopathologist attend lymphoma clinic and meet with patients in order to familiarize them with the diagnostic process, show them their (imaged) biopsy material, and answer any questions.

Design: The lymphoma clinic coordinator approached patients in order to determine whether they would be interested in meeting with the hematopathologist. This service was only available to patients whose pathology had been reviewed. A handout was provided describing what a pathologist does and some of the questions that might be appropriate. The pathologist's name and contact information (cell phone number and email address) were provided. If the patient was interested, the coordinator would alert the pathologist. At the first morning clinic, the pathologist brought a double-headed microscope and glass slides for review. As this proved cumbersome, scanned images and a laptop computer were used in subsequent clinics. After the visit, the patients were asked to anonymously complete a questionnaire to determine whether patients felt that meeting with the pathologist was valuable. The attending H/O was also surveyed to determine whether this was helpful for them.

Results: The hematopathologist attended 4 lymphoma clinics. All patients expressed an interest in meeting with the pathologist. 16 patients were seen. The pathologist met with the patient after the physician assistant or resident. 100% of patients who completed the survey (14) "found this meeting helpful in understanding the diagnosis" and would "recommend this experience" to other patients. The H/O clinicians were also surveyed. Three out of four responded to the survey. The resondents found it helpful as it allowed them to ask the pathologist questions about the pathology before seeing the patient. It was also believed to facilitate patient education.

Conclusions: Pathology and other areas of clinical medicine need to find ways to add value in a new era of personalized care and new payment models. This pilot project suggests that patients and physicians find it helpful to have a pathologist present in clinic to answer questions and review the diagnostic material. Based on the results of this study, efforts are being made to implement this model at our academic cancer center.

2009 Her2/neu Testing in Breast Cancer: Comparative Analysis of In Situ Hybridization and Immunohistochemistry in a Dual-Testing System

James Solomon, Oluwole Fadare, Farnaz Hasteh. UCSD, San Diego, CA.

Background: HER2/neu status is an important predictive and prognostic factor in breast cancer, and its accurate determination is critical for planning treatment. According to the newest ASCO/CAP guidelines, either immunohistochemistry (IHC) or FISH may be used to determine HER2 status. There is currently no consensus on the most appropriate testing algorithm, and both tests may be significantly affected by pre-analytic and analytic factors. At our institution, HER2 is tested by both IHC and FISH on all newly diagnosed breast cancers to ensure that no HER2 positive patients are missed. Here, we seek to determine how frequently HER2-amplified tumors would be missed if testing were restricted to one modality by assessing HER2 status concordance as determined by FISH and IHC and by analyzing modality-discordant cases.

Design: Consecutive cases of invasive breast cancer newly diagnosed at a tertiary care academic medical center between June 2014 and July 2015 were assessed, and HER2 status was determined by both IHC and FISH. FISH was performed using dual-color HER2/CEP17 probes, and if equivocal results were obtained, reflex testing using HER2/LIS1 probes was used. HER2 IHC was performed on a Ventana automated platform (rabbit monoclonal antibody, clone 4B5, Roche/Ventana, AZ). Results from both modalities were scored and reported using ASCO/CAP 2013 criteria.

Results: We assessed 251 separate breast cancers from 238 patients. Overall, 18.3% were positive for HER2 by either modality. Respectively, 41%, 22%, 28% and 8.4% of cases scored 0, 1+, 2+, and 3+ by IHC. By FISH, 18% were HER-2 amplified, and 5% were equivocal. The concordance between IHC and FISH testing was 96.8%. Seven of the 238 patients (2.9%) were reclassified as being HER2 positive after a negative IHC result (ASCO/CAP 2013 value of 0 or 1+), while only one case with a score of 3+ on IHC was found to be HER2-non-amplified by FISH. Thus, if a reflex strategy were adopted based on IHC (i.e. only performing HER2 FISH for cases with equivocal [2+] IHC results), 15% of all HER2-amplified cases would have been missed.

Conclusions: There continues to be a small but persistent discrepancy rate between HER2 status as determined by IHC and FISH in routine practice. At our institution, the discrepancy appears to be primary centered on cases scored 0-1+ by IHC. Therefore, a reflex strategy based on IHC results may potentially deny a small cohort of patients needed therapy. These factors should be considered as testing guidelines are formulated and the cost-benefit analyses of various testing approaches are assessed.

2010 Quality Assurance Protocols for Breast and Gynecologic Pathology Practice in a CLIA Laboratory Minimize Serious Events and Maximize Patient Safety

Catherine Stoos, Rohit Bhargava, Gloria J Carter, Abbie Mallon, David Dabbs. Magee Womens Hospital, Pittsburgh, PA.

Background: Surgical pathologic diagnoses direct patient management, and therefore correct diagnostic interpretation is essential for proper patient management. Some recent published data on diagnostic agreements among pathologists in a research setting have alarmed the public, but these studies do not reflect the real world of pathology practice. Laboratory accreditation specifies the necessity of a quality assurance program compliant with CLIA '88, under the aegis of the Medical Director. Quality assurance (QA) protocols are one tool pathologists use to increase diagnostic accuracy, reduce error and maximize patient safety. The goal of this study is to review our quality metrics to determine whether our protocols minimize serious events that might alter patient management.

Design: Amended reports for breast and GYN surgical pathology specimens from 2012 to 2014 were reviewed. Amended reports are categorized as follows: A - minor disagreement, such as a spelling error which had no bearing on patient care; B - moderate disagreement, including defects in diagnoses without effect on patient care, and; C - major disagreement, which include major discrepancies affecting the diagnosis and treatment of a patient. The departmental quality assurance protocol metrics, which are monitored by a committee with CLIA Medical Director as chair, include 10% random review; frozen section vs final diagnosis; tumor board reviews; consult reviews; double reads of all breast core biopsies; double reads of all new malignancies; concurrent review of biopsy material with surgical resections, and cytohistologic correlations.

Results: Of the 63,665 breast and GYN specimen reports created from 2012 to 2014, 343 (0.54%) required amendment due to a QA metric-discovered discrepancy. The most common reason for amendment within both breast and GYN specimens was type A, or minor disagreement (amended for type A discrepancy: 78.7% of total; 81.9% of breast; 72.6% of GYN). Type B, or moderate disagreement discrepancies, accounted for 21.3% of all amended cases (amended for type B discrepancy: 18.1% of breast; 27.3% of GYN). Of all breast and GYN reports reviewed, there were no amended cases which were categorized as type C, or major disagreements which would affect patient treatment.

Conclusions: Our QA data show that, when pathologists practice QA targeted protocols designed to reach concordance and minimize diagnostic error, the occurrence of major diagnostic disagreements that could affect patient management for breast or gynecologic pathology diagnoses are distinctly uncommon.

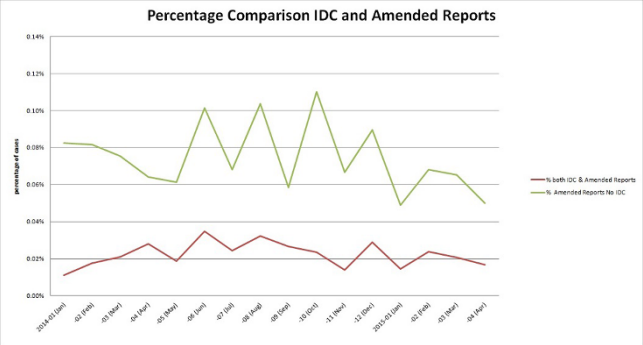
2011 Impact of Intradepartmental Consultation on Amended Report Rate; Findings from the Irish National Quality Improvement Programme in Histopathology

Niall Swan, Philip Ryan, Kieran Sheahan, Ann Treacy, Sine Phelan, Julie McCarthy, Mairead Guinan, John Conor O'Keane. Royal College of Physicians of Ireland, Dublin, Ireland.

Background: Intradepartmental consultation (IDC) is an important quality activity that occurs prior to report authorisation and forms an integral component of a laboratory quality improvement (QI) programme with the ultimate aim of reducing the interpretive error rate of pathology reports. While quality assurance guidelines propose a 10% IDC rate this standard has not been validated and the impact of IDC has not been proven on a large scale review. Since 2009 Irish histopathology laboratories have participated in a National QI Programme where specimen-based QI coded data is electronically submitted to a central database which incorporates standardised definitions for IDC and amended report. The aim of this review was to establish if IDC reduces the amended report rate at a national level.

Design: Cases submitted to the Irish National QI Programme over a 16 month period (January 2014 - April 2015) were identified using the National Quality Assurance Intelligence System (NQAIS), a novel information technology system designed to generate national quality pathology metrics. Cases coded as containing an amended report and IDC review were extracted and the data was analysed using SPSS with a Pearson Chi Square test to assess significance of association.

Results: A total of 537,590 histology and cytology cases were submitted by 33 laboratories to the NQAIS over 16 months with 31,925 subjected to IDC (mean = 5.9%). The total number of amended reports was 525 (mean = 0.097%) with 405 (77%) not subjected to IDC. The amended report rate for cases subjected to IDC was 0.037%, a significant reduction when compared to cases not subjected to IDC (p value = 0.022).



Conclusions: Intradepartmental consultation is effective in reducing the number of amended reports even when the rate of review is below the recommended standard of 10%. However despite prospective review interpretive errors still occur and other forms of peer review including targeted retrospective review and multidisciplinary team / tumour board meetings are needed to further reduce the rate of amended reports.

2012 Communication with Clinicians in Anatomical Pathology

Ann Treacy, John Conor O'Keane, Julie McCarthy, Sine Phelan, Howard Johnson, Jennifer Martin, Mairead Guinan, Philip Ryan, Niall Swan, Kieran Sheahan. Royal College of Physicians of Ireland, Dublin, Ireland; HSE, Dublin, Ireland.

Background: Communication between pathologists and our clinical colleagues is an important component of professional practice. The communication of critical diagnoses and the obtaining of additional clinical information are common reasons for this activity, however little data is available assessing this activity.

Design: The Irish National Quality Improvement (QI) Programme in Histopathology commenced in January 2009 and is led by the Faculty of Pathology Royal College of Physicians of Ireland (RCPI). The aim of the Programme is to provide a framework that enhances patient safety with timely, accurate and complete pathological diagnoses. The QI Programme has published guidelines setting out key quality indicators including communication of reports to clinicians. We sought to quantify and evaluate the use of this parameter. Review of the national data over a 16-month period was carried out along with audit of the use of a specific Q code (Q23) in 4 centres enrolled in the QI programme with emphasis on specimen type, diagnosis and reason for communication.

Results: Over a 16-month period (January 2014 – April 2015) 9453 of 537590 cases (1.8%) were coded as Q23 (range 1.5 – 2.1%). Non-designated cancer centres communicated with clinicians more often (2%, range 0.6-13.5%) than designated cancer centres (1.6%, range 0.6-2.7%). Data from the 4 centres audited showed the most frequent reasons for communicating with clinicians were urgent specimens 28%, malignant diagnoses 13.7%, unsuspected diagnoses 7.5% and clinician request 7.5%. The specific reasons stated for communication with clinicians from the 4 centres audited were as follows;

Reasons for communication	Hospital 1	Hospital 2	Hospital 3	Hospital 4
Change in diagnosis		2	2	
Clinical concern re malignancy	6		4	
Clinical information required			12	
Clinician request		1	26	3
Clinicopathological Correlation		4	10	1
Complex case	12		3	
Malignant diagnosis	50		5	
Medical emergency	9		5	
Non diagnostic	2	1		
Protocol		25	7	
Suspicious for malignancy	8		1	
Technical issue	1		1	
Unknown	5		1	42
Unsuspected diagnosis	6	6	8	10
Unusual diagnosis			6	
Labelled Urgent	1	61	9	44

Conclusions: Pathologists communicate with clinicians directly for a wide variety of reasons, which underlines the vital role of this quality indicator in patient safety. A marked variation exists in Irish hospitals with respect to the frequency and the underlying initiating factor for the communication. These findings present an opportunity for review of current communication practice and to consider quality improvements with a view to standardization of critical diagnosis reporting.

2013 Tumor Heterogeneity Compromises Studies on the Effect of Prolonged Fixation on Immunohistochemistry (IHC) Reporting of Breast Biomarkers

Tra Truong, Ken Kao, LUIS Gai, Kim Voisey. Memorial University, St John's, Newfoundland, Canada.

Background: Fixation is the key step of the preanalytical process that affects both turn around time and quality of IHC reporting. Previous studies claim that prolonged fixation in 10% neutral-buffered formalin (NBF) does not affect IHC staining of ER, PR and HER-2 for invasive breast cancer. Nonetheless, 24 to 72 hour fixation of breast specimens in NBF at room temperature is recommended for optimal biomarker IHC results. Our study examines the effects of prolonged fixation beyond 72 hours up to 336 hours on the IHC results at our center.

Design: Submitted invasive breast tumor resections with volumes permitting additional sampling were selected for our study. Sections submitted for diagnosis served as controls for the study of the corresponding breast tumor. Cross-sections of remaining unsubmitted tumors were divided and subjected to different fixation times (up to 336 ± 2hours) in NBF. They were subsequently processed using validated routine procedures in our lab. ER, PR and HER-2 immunostaining was performed using validated protocols. Quality of H&E and IHC stains for Tissue Microarrays and Whole-tissue sections were evaluated independently by 2 pathologists blinded from the variable fixation durations. An average score, 0-8 for ER, PR and 0-3 for HER-2, is listed as the final result.

Results: Breast tumor characteristics

Breast ID	Diagnosis	Tumor size (cm)	Grade	Stage
1	Invasive Carcinoma with Ductal and Lobular features	8.20	3	T1N1a
2	Invasive Ductal Carcinoma	2.90	2	T4bN0
3	Invasive Ductal Carcinoma	3.00	3	T2N1a
4	Invasive Lobular Carcinoma, Pleomorphic subtype	3.60	2	T2N2a

Mean Biomarker IHC Scores in breast tumors post 242 to 336±2h of fixation

Tissue construction		Tissue Microarrays		Whole Tissues	
Breast ID		1	2	3	4
Number of controls		12 cores	8 cores	1 section	1 section
Number of tests		16 cores	12 cores	4 sections	4 sections
Reported ER(SP1)		Uniformly (+) 98%	Uniformly (+) 100%	Uniformly (+) 100%	Variably (+) 30%
ER (SP1)	control	7.67	8.00	8.00	6.00
	242 to 336±2h	7.25	8.00	8.00	4.50
Reported PR(16)		Variably (+) 20%	Uniformly (+) 100%	Uniformly (+) 100%	Negative (0)0%
PR(IE2)	control	5.67	8.00	8.00	0
	242 to 336±2h	6.00	8.00	8.00	0
Reported HER2(SP3)		Positive (3+)100%	Negative (0)0%	Negative (1+)N/A	Equivocal (2+)50%
HER2(45B)	control	2.75	2.00	0.75	0.25
	242 to 336±2h	3.00	2.00	2.00	2.00

Conclusions: Our preliminary results demonstrate prolonged fixation up to 14 days did not significantly affect the IHC result in resected breast tumors with high score (score 7-8) ER, PR and (score 3+) HER-2 compared to the controls, regardless of tumor characteristics. Biomarkers of low-intermediate scores, however, showed a high degree of intratumoral heterogeneity in receptor staining which would affect study conclusion on the effect of preanalytical variability on IHC reporting.

2014 Can Westgard Rules Identify Trends in Nondiagnostic Rates for Thyroid Fine Needle Aspiration?

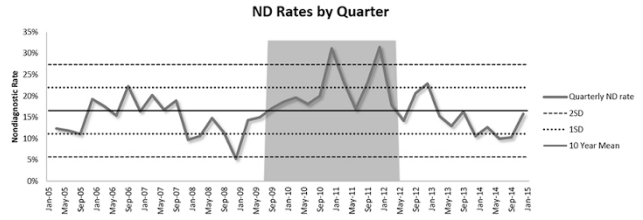
Janice Tyler, Jeffrey Goldsmith, Pamela Hartzband, James V Hennessey, Michiya Nishino. Beth Israel Deaconess Medical Center, Boston, MA; Harvard Medical School, Boston, MA.

Background: Between 5-20% of thyroid fine needle aspirations (FNA) are nondiagnostic (ND), presenting management challenges for patients and clinicians. ND FNAs may be due to intrinsic properties of the thyroid nodule as well as extrinsic factors such as operator experience, processing artifact, and cytopathologists' thresholds for considering specimens sufficiently cellular for diagnosis. To our knowledge, it is unknown whether systematic monitoring of ND thyroid FNA rates over time (similar to Levey-Jennings plots of control values) and application of Westgard rules would be a useful quality control method for identifying factors contributing to changes in the ND rate.

Design: Our cytopathology pathology database was retrospectively searched for thyroid FNAs performed between 1/2005 and 7/2015. The rate of ND thyroid FNAs was calculated for each quarter. Westgard rules were applied, and trends were correlated with changes in practice that may have influenced ND rates.

Results: Over the 10-year study period, 7984 thyroid FNA samples were identified, of which 1302 were considered ND (16.3%). Quarterly ND rates ranged from 5.3% to 31.5%, with a mean of 16.6% and standard deviation of 5.4% (Figure 1). Westgard rules identified a 10:mean rule violation (i.e., 10 consecutive measurements on the same side of the mean) between Q3 2009 to Q1 2012, correlating roughly to the timeframe when a standardized reporting system for thyroid FNA (Bethesda System) was implemented. No violations of the 1:3s, 2:2s, or 4:1s Westgard rules were noted.

Conclusions: Quarterly ND rates for thyroid FNAs are subject to a high degree of variability, limiting its utility in identifying actionable events that influence short-term deviation from a target ND rate. However, application of Westgard rules may help identify longer-term trends in ND rates for thyroid FNAs.



2015 A Quantitative Method to Measure Pathology Assistants' Productivity Using Standardized Relative Value Units

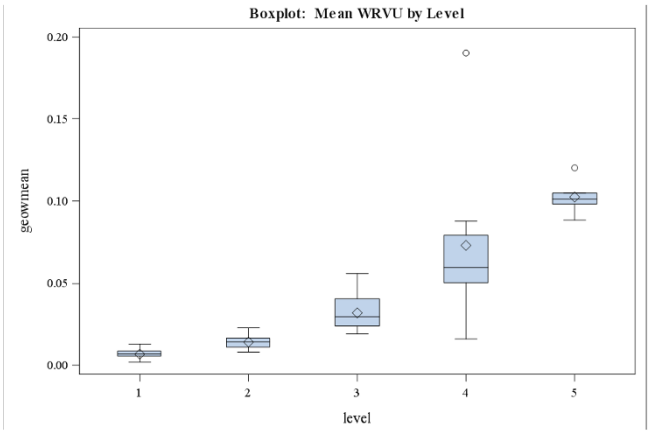
Vladimir H Volel, Tarush Kothari, Raheela Querishi, Diane Groppi, Mike Raugnath, Claudine Alexis, Tylis Y Chang, Oksana Yaskiv, James M Crawford, Tawfiqul Bhuiya. North Shore - LIJ Health System, Lake Success, NY.

Background: A validated and objective method to quantify Pathology Assistants (PA) productivity does not exist. Standardized relative value units based on complexity of diagnosis are commonly used for measuring Pathologists' productivity. However this method is not suitable for measuring PA productivity as it does not account for specimen complexity and workload. We describe a rigorous method to calculate the standardized relative value unit of work-effort (wRVU) required to dissect any pathology specimen.

Design: We collected data on processing times for 11,933 specimens over a 4 month period (6/30/14-10/30/14) at our 2 tertiary academic medical centers. This included 158 pathology specimen descriptors encompassing CPT codes 88300 to 88309. Processing times were converted into a wRVU by dividing by 420 (minutes in a 7 hour working day). A priori we divided all the CPT descriptors into 5 distinct categories based on the time taken to dissect a specimen. The data were log transformed to obtain normal distributions. For all CPT descriptors the weighted geometric mean wRVU, standard error and 95% confidence intervals were calculated.

Results: There was a significant difference in the geometric mean wRVU by level ($p<0.0001$). There was no disagreement between the levels assigned a priori and the levels based on calculated mean wRVU, suggesting that our categorization based on total time for dissection is both practical and objective.

Level	Minutes	Number of CPT Descriptors	Geometric Mean	Standard Deviation	Minimum	Maximum
1	<6	74	0.0069	0.0022	0.0020	0.0129
2	6-12	38	0.0141	0.0038	0.0079	0.0229
3	13-30	32	0.0322	0.0100	0.0189	0.0561
4	31-45	9	0.0727	0.0492	0.0159	0.1900
5	>45	5	0.1025	0.0117	0.0882	0.1204



Conclusions: We propose an objective and quantitative method to measure PA productivity which accounts for specimen complexity. This tool facilitates the comparison of practical capacity to previously undefined theoretical capacity. We now have the ability to measure productivity of the PA workforce and track performance improvement efforts to remove inefficiencies in the production process.

2016 Evaluation of Repeated Biomarker Testing In a Breast Consultation Service: One Institution Experience

Elena Vrotsos, Cesar A Llanos. University of Miami, Miami, FL.

Background: Detailed attention to test utilization by Medicare and other payers as a way to curb health care cost is on the rise. This could translate in decreased reimbursements in a field where test pricing is already on the fall. At our institution, we routinely repeat receptor studies on all consult cases. In this study we evaluated the concordance of repeated tests and proposed more clinically significant and cost-beneficial approach.

Design: We performed an exploratory retrospective review of 100 neoplastic breast consult cases. We studied the percentage of concordance of the initial ER, PR and HER-2 testing with our repeated "in house" tests results, evaluated the medical necessity of these repeated tests and analyzed the possible clinical impact of discordant results.

Results: Out of the 100 cases, 91 were invasive carcinoma and 9 carcinoma in situ (DCIS). The concordance rate was 100% and 89% for ER and PR respectively in the DCIS group. In the invasive carcinoma group there was 96.7% and 95.6% concordance rate for ER and for PR respectively.

However, Her-2 concordance rate was 78%. Twenty cases were discordant for Her-2. Out of the twenty cases 6 cases had a major discordance with clinical significance (positive to negative or vice versa). In the six discordant cases, two cases were negative by IHC in the outside institution and positive in our institution with conformation by FISH. The other four major discordant cases were called positive by IHC outside, and were negative by IHC and FISH in our institution.

Conclusions: There is high concordance between ER and PR results, hence repeat testing may not be necessary. Moreover, this high concordance can potentially justify no repeated biomarker testing in cases of DCIS or invasive carcinoma. This will potentially decrease test overutilization and overall cost.

We identified a discordant rate of 21.9% in HER-2 testing, these discordant results have clinically significant impact with costly therapeutic implications. In this scenario, repeated testing with immunohistochemistry with possibly reflex to FISH or directly with FISH may be justified.

Immunohistochemistry and FISH testing combined can amount to close to 900 to 1200 dollars per case. In our institution, we receive approximately 400 cases a year. Reduced health care cost with a utilization measure like the one proposed here can amount to close to $360.000 per year.

2017 Laboratory Requisition Forms Often Lack Critical Information: An Initiative to Improve Communication and Enhance Patient Safety at The Ottawa Hospital

Jason K Wasserman, Iris Teo, Darren Tse, John Veinot, Bibianna Purgina. University of Ottawa, Ottawa, ON, Canada.

Background: Every specimen submitted for pathological analysis is accompanied by a laboratory requisition form (LRF). Despite the fact that this LRF is often the only documentation provided to the pathologist, many lack critical information. As part of an initiative to enhance patient safety, we sought to identify deficiencies in the current LRF and devise a new LRF to improve communication between clinicians and pathologists.

Design: The Anatomical Pathology database was searched for all cases accessioned in 2014. Mandatory fields in our LRF were assessed including: specimen site, collection time and date, most responsible physician (MRP), physician signature, and clinical history. In 100 consecutive finalized cases the 'clinical history' provided was reviewed and compared with the patient's electronic medical record for completeness and accuracy. A revised LRF was drafted to address deficiencies identified in the audit.

Results: 51,722 cases were accessioned: 273 (0.5%) were missing anatomical site/laterality, 739 (1.4%) were missing the collection date and time, and 6031 (12%) did not include a clinical history. Of the 100 LRF reviewed, only 25% included all mandatory information; fifteen requisitions did not include any clinical information and in 18 cases the history was incomplete. After 6 months in circulation, there was a significant increase in the percentage of LRF that included all mandatory data (71% vs 25%; $p<0.001$) although 15% were still lacking any clinical information.

Conclusions: Very few LRF are submitted complete. Collaborating with our clinical colleagues, we drafted a new LRF which made it easier to communicate critical information including time in formalin and key aspects of the clinical history. However, in an academic center with high turnover of trainees and staff, constant communication and education is critical to ensure a properly completed LRF.

2018 Utility of Routine Histopathological Analysis Following Orthopaedic Procedures for Benign Indications

Anna-Sophie Weidner, Francesca Khani, Theresa Scognamiglio. Weill Cornell Medical College, New York, NY.

Background: At many institutions, it is standard practice to histologically assess the products of all orthopaedic procedures, even those performed for benign indications such as hallux abducto valgus deformity (bunion) and meniscal tear. To our knowledge, the frequency of clinically significant findings in these specimens has not been reported.

Design: We reviewed the reports of 441 consecutive bunionectomy and 68 meniscectomy cases performed between July 2005 and July 2015 through a search of our pathology database. Clinical information was obtained through the electronic medical record. Any abnormal histopathological findings were correlated with the clinical impression. We assessed the approximate annual billing charges associated with the processing and evaluation of these specimens.

Results: No occult malignancy was detected in any bunion or meniscal resection specimen. Benign lesions identified in bunionectomy cases included neuroma (4/441), ganglion cyst (6/441), synovial cyst (1/441), dematofibroma (1/441), and lentigo (1/441) and in meniscectomy cases included ganglion cyst (1/68); in all cases, the presence of an abnormal finding was clinically apparent and communicated to the pathology department. In 2 bunionectomy specimens and 1 meniscectomy specimen, crystalline deposits consistent with gout or pseudogout were identified, and synovial osteochondromatosis was present in 1 bunion resection; it is unknown whether these represent unexpected findings as the clinical information was unavailable for review. The approximate billing charges for bunion and meniscus resections at our institution amounted to over $10,000 annually.

Conclusions: Routine histopathological evaluation of bunionectomy and meniscal resection specimens is unnecessary in the absence of clinically apparent abnormal findings. Elimination of this practice would be cost effective.

2019 Intraoperative Evaluation of Borderline Cystic Ovarian Lesions: Are We "at Least" Concordant with the Final Diagnosis?

Kyle J White, Andre Pinto. University of Miami / Jackson Memorial Hospital, Miami, FL.

Background: Benign and borderline ("low malignant potential") cystic ovarian lesions are usually challenging specimens when submitted for intraoperative evaluation. Many factors can contribute to a discordant diagnosis of invasive carcinoma at the time of frozen section (FS), and they include sampling of key areas, number of sections taken, and experience of the pathologist. Although the limited value of this technique is relatively well known, a discrepant diagnosis from the time of intraoperative assessment (i.e., calling a borderline tumor invasive carcinoma) can lead to treatment implications.

Design: We evaluated the data of all reports from lesions classified as borderline tumors (BT) at the time of FS, of a 12-year period, from our pathology database. The reports were then compared with the final pathology. They included borderline tumors of serous (SBT), mucinous (MBT) and endometrioid (EBT) morphology. The number of sections taken for FS was also obtained, and correlated with the results.

Results: A total of 82 reports were retrieved. They consisted of (based on FS diagnosis) 42 SBT, 23 MBT and 17 BTs (with no subtype specified). Among these, a total of 9 (11%) had the diagnosis revised (presence of invasive adenocarcinoma on permanent sections- see table). Six (6) other cases had only differences in the epithelial subtype, of no clinical significance.

Interestingly, on 7/9 (77%) of the cases on which a malignant component was subsequently found contained notes in the FS report that suggested the concern for the potential presence of a (non-sampled) malignancy. The most used expression was "at least borderline".

The median sections taken at FS within the non-discordant group was 2, compared to a median of 2.5 sections within the discordant group (distribution difference not significant; Wilcox Rank Sum test, p=0.1766).

According to chart review (when information was available), only three patients (3.6% of total) required a second surgical intervention, among all subjects with BTs.

Conclusions: Intraoperative evaluation of ovarian borderline epithelial lesions, although challenging, seems to be reliable. In our cohort, 11% of cases had a diagnostic change upon extensive sampling for permanent sections. According to chart review, 3.6% of patients required a subsequent surgical procedure. We infer that, even in discordant cases, the communication of potential concerns between the pathologist and the surgeon, as well as understanding the limitations of this technique in such tumors, may explain the low impact on patient care.

2020 A Proficiency Testing Scheme for IDH1 R132H IHC: The cIQc Experience

Jennifer Won, Max Signaevski, John Garratt, Emina Torlakovic, Blake Gilks, Stephen Yip. Canadian Immunohistochemistry Quality Control (cIQc), Vancouver, Canada; University of British Columbia, Vancouver, Canada; Vancouver General Hospital, Vancouver, Canada.

Background: Recurrent mutations in *IDH1/2* are found in infiltrative gliomas. Present in nearly all cases of secondary glioblastoma arising from low-grade gliomas, they are rarely in the primary glioblastoma. *IDH1/2* mutations are present in 60–80% of WHO grade II and III astrocytomas and oligodendrogliomas, but absent in non-neoplastic lesions that can mimic tumors. Non-infiltrative gliomas, including pilocytic astrocytoma, dysembryoplastic neuroepithelial tumor, and ganglioglioma, do not contain *IDH1/2* mutations. Missense mutation causing an arginine to histidine change in codon 132 (R132H) is most common, accounting for ~90% of *IDH1* mutations in glioma. IHC for the mutant *IDH1* R132H protein provides an essential adjunct in diagnostic neuropathology by increasing diagnostic confidence. This is particularly helpful in cases with presence of histologically-atypical cells of unknown etiology and limited availability of diagnostic tissue, such as brain biopsies, where spatial heterogeneity may result in neoplastic cells admixed with reactive, non-neoplastic cells.

Design: The cIQc has performed two *IDH1* R132H IHC proficiency testing runs, in which participants stained a tissue microarray with 28 gliomas and non-neoplastic lesions previously subjected to Sanger sequencing for *IDH1* and *IDH2* hot spot mutations. 16 and 18 labs participated in Run 1 and 2, respectively. Results were self-reported online in cIQc TMA Scorer then revised by cIQc if needed during an expert assessment meeting. Percent concordance with sequencing and Cohen's kappa values were calculated for which >90% and >0.80, respectively, were considered an acceptable result.

Results: For Run 1, 12 of 16 (75%) labs had acceptable staining based on described criteria. For Run 2, 16 of 18 (89%) labs passed the cIQc proficiency testing criteria. The increase in pass rate was not statistically significant (p=0.29).

	Run 1		Run 2	
Lab ID	Concordance with sequencing	Cohen's kappa	Concordance with sequencing	Cohen's kappa
101	86%	0.73	100%	1
102	100%	1	100%	1
103	100%	1	100%	1
107	100%	1	100%	1
110	67%	0.35	100%	1
111	86%	0.73	87%	0.74
112	95%	0.91	100%	1
114	100%	1	100%	1
123	100%	1	100%	1
125	100%	1	100%	1
126	100%	1	100%	1
149	100%	1	96%	0.93
162	100%	1	96%	0.92
175	100%	1	100%	1
191	100%	1	96%	0.92
202	86%	0.73	100%	1
-	-	-	100%	1
-	-	-	89%	0.78

Conclusions: While the increase in pass rate was not statically significant, most underperforming labs in Run 1 achieved markedly improved results in Run 2 after protocol optimization following cIQc feedback, demonstrating the value of continued participation in proficiency testing schemes.

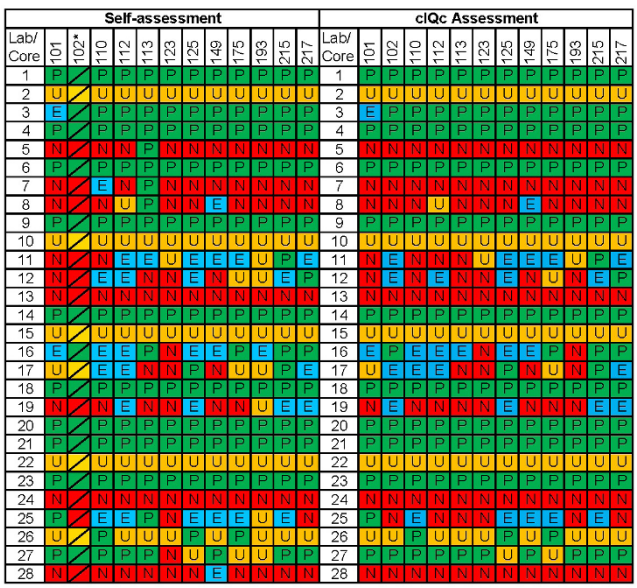
2021 Quality Assurance of ATRX Immunohistochemistry in a Multi-Centre Diagnostic Neuropathology Initiative

Jennifer Won, John Garratt, Emina Torlakovic, Blake Gilks, Stephen Yip. Canadian Immunohistochemistry Quality Control (cIQc), Vancouver, Canada; University of British Columbia, Vancouver, Canada; Vancouver General Hospital, Vancouver, Canada.

Background: Combined application of IDH1 R132H and ATRX immunohistochemistry plus 1p/19q co-deletion analysis can significantly increase the diagnostic and prognostic accuracy of low grade gliomas. Constituting a key parameter in this integrated diagnosis, abrogated ATRX protein expression based on immunohistochemistry is used as a surrogate for *ATRX* mutation, which is strongly associated with *IDH1/2* mutated astrocytomas and not oligodendrogliomas. ATRX immunohistochemistry can refine the diagnostic accuracy of low grade glioma; however, it is heavily influenced by the quality of tissue material. Interpretation is also particularly challenging as nuclear positivity is seen in endothelial cells, entrapped neurons, microglia and reactive astrocytes.

Design: An inaugural ATRX immunohistochemistry proficiency testing challenge was administered by the cIQc in June 2015. 12 laboratories were asked to stain a tissue microarray consisting of 28 single-core gliomas and non-neoplastic lesions that have been previously subjected to molecular analyses for *IDH1/2* mutations and 1p/19q co-deletion analysis for select cases. Results from ATRX stained slides were first self-reported online in the cIQc TMA Scorer website then revised, if necessary, by the cIQc during an expert assessment meeting. The same TMA was previously sent out to many of the same laboratories for proficiency testing of IDH1 R132H immunohistochemistry.

Results: A high degree of sensitivity and specificity was observed for ATRX immunohistochemistry on glioma cases despite a wide spectrum of background staining by participating laboratories. Staining and interpretation of non-glioma (e.g. cavernoma or gliosis) tissue cores were more variable.



Conclusions: ATRX is a challenging immunohistochemical marker. Interpretation must be done by an experienced neuropathologist and continued participation in external quality assurance is necessary. When correctly interpreted, ATRX immunohistochemistry is valuable as a subsequent test for distinguishing between astrocytomas and oligodendrogliomas after IDH1 R132H immunohistochemistry has been performed for differential diagnosis of glioma.

2022 Quick Review of Fine Needle Aspiration Accuracy with Focus on Cytohistologic Correlation and Evaluation of Discrepant Cases in a Limited Sample

Somaye Yekezare, Niloufar Reisian, Xiaoyan Liao, Farnaz Hasteh. University of California San Diego Health System, San Diego, CA; Kaiser Medical Center, San Diego, CA.

Background: Fine needle aspiration biopsy (FNAB) is a minimally invasive procedure commonly utilized for the primary investigation of mass lesions. Correlation of FNAB diagnosis with histopathologic results is part of quality assurance and quality control in cytology laboratories. The aim of this study was to investigate the diagnostic performance of FNAB and to identify the specimen types that are prone to errors.

Design: A total of 1125 FNABs performed at our institutes in 2013 were selected and 401 satisfactory cases with the follow-up surgical specimens were included for this study. FNAB results were classified as negative, atypia, or positive; whereas final pathologic diagnoses were classified as malignant or benign. The cases were categorized into lymph nodes (n=124), lung and chest (n=71), GI tract and liver (n=58), pancreatobiliary tract (n=45), thyroid (n=42), head and neck (n=34), bone and soft tissue (n=17), and genitourinary (n=10). Fifteen (3.7%) cases with discordant diagnosis on follow up samples were identified. The discrepant cases were reviewed by two cytopathologists blindly and their diagnoses were compared to the original results. Discrepancies of these cases were classified as interpretive or possibly sampling errors.

Results: Cytology diagnosis was confirmed by histology in 386 of 401 FNABs (96.3%). Only 15 cases were discrepant and review of these cases showed 9 cases were misdiagnosed originally (interpretation errors 60%). The remaining 6 cases were diagnosed correctly and did not represent the lesion, most likely due to sampling error (40%). Thyroid (9.5%) followed by pancreaticobiliary tract (6.6%) had the highest interpretive discrepancy rates; whereas head and neck accounted for the highest sampling errors (5.8%). Fisher's exact test yielded no significant differences in discrepancy rates for different specimen types (P>0.05). Among the 15 discrepant cases, 11 were undercalled as benign or atypia (80%) and three were overcalled as positive (20%). Only 3 cases with interpretive errors were reviewed by other pathologists as a second opinion (33%).

Conclusions: FNAB has an excellent correlation with surgical pathology results and is an effective procedure for the diagnosis of mass lesions. The accuracy of this highly reliable tool can be further improved with accurate sampling. Interpretive errors can be minimized by careful examination of the cytologic findings and intradepartmental consultation.

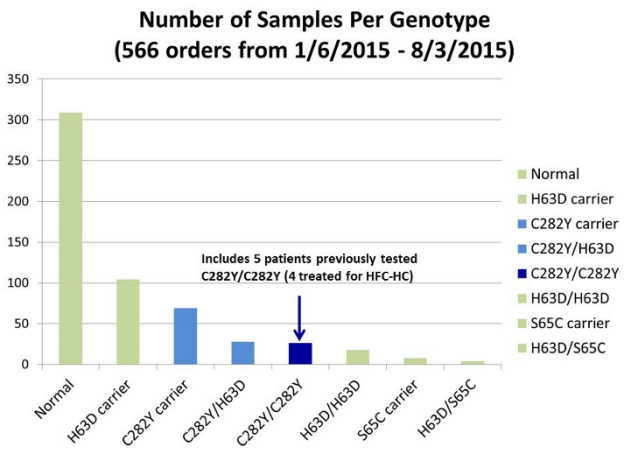
2023 Improving Utilization of Hemochromatosis Testing

Yaolin Zhou, Gary Procop, Jacquelyn Riley. Cleveland Clinic, Cleveland, OH.

Background: HFE hemochromatosis (HFE-HC) due to homozygous C282Y mutations in the *HFE* gene is a well-known cause of inherited iron overload syndrome, yet disease penetrance is low and iron studies are non-specific. We integrated international practice guidelines with our clinical data for the evaluation of patients with suspected HFE-HC.

Design: To assess routine practice at our institution, we performed a retrospective review of 566 *HFE* orders and a subset analysis of 55 consecutive orders and all HFE-HC patients. These were compared with European guidelines, which encourage step-wise evaluation of hyperferritinemia, and U.S. guidelines, which recommend genetic testing of patients with abnormal iron studies or a positive family history.

Results: Results from 1/6/2015 to 8/3/2015 showed 4.6% C282Y/C282Y, 12% C282Y carriers, 5% C282Y/H63D, and 77% non-risk genotypes. Only C282Y/C282Y patients have significant risk for iron overload. Five HFE-HC patients had a positive family history. At least 7 tests were ordered on known HFE-HC patients (new diagnosis rate <3.5%).



Of 43 *HFE* orders from 7/20-8/3/2015, gastroenterologists and generalists ordered tests most frequently. Seventeen patients had liver disease, e.g. cirrhosis (6), viral hepatitis (5), fatty liver (4), acute alcohol intoxication (2) and transfusion-related iron overload (2). Patients with non-risk genotypes had lower mean serum ferritin (613 ng/mL vs. 1,070 ng/mL, $p=0.03$) and transferrin saturation (43% vs. 66%, $p<0.01$) than those with C282Y genotypes. The recommended criterion of TS>45% was 94% sensitive in identifying patients with C282Y and 100% sensitive for C282Y/C282Y. Serum ferritin>1,000 mcg/L, which correlates with cirrhosis, was 76% specific for C282Y. Most C282Y heterozygotes (4) and C282Y/C282Y (13) patients with available results met these cut offs, and two C282Y patients met criteria based on family history alone.

Conclusions: The proposed criteria of TS>45%, ferritin>1,000 mcg/L, or a positive family history, was 100% sensitive and moderately specific for C282Y screening. Our institution is now considering an approach to include measuring ferritin/TS, and reflexive *HFE* gene testing only when criteria are met. If used broadly, the algorithm should detect all at-risk patients and result in an estimated 43% reduction of unnecessary *HFE* tests and a lower cost per new diagnosis.

Techniques (including Ultrastructure)

2024 Complementary Value of Electron Microscopy and Immunohistochemistry in the Diagnosis of Non-Small Cell Lung Cancer

Raquel Albero, Lara Pijuan, Mercedes Simon, Nuria Juanpere, Ivonne Vazquez, Adria Lloret, Jessica Munne, Ignacio Sanchez, Sergio Serrano, Josep Lloreta. Hospital del Mar, Barcelona, Spain; Istituto Clinico Humanitas, Milan, Italy; Autonomous University of Barcelona, Barcelona, Spain; Pompeu Fabra University, Barcelona, Spain.

Background: Pathological classification of lung cancer has been redefined by cytogenetics and molecular data. Consequently, therapeutic targets have been identified for pulmonary adenocarcinoma (ADK). Thus, it is crucial to accurately distinguish between ADK and squamous cell carcinoma (SQCC) in poorly differentiated cases. Immunohistochemistry (IHC) is very helpful in making this differential diagnosis. However, a subset of cases remains classified as Non-Small Cell Lung Carcinoma, NOS (NSCLC-NOS), after IHC. In these cases, Electron Microscopy (EM) can be a useful tool, as it objectively identifies glandular differentiation. The aim of this study was to determine the value of EM and IHC in the NSCLC-NOS subclassification.

Design: Forty-eight NSCLC-NOS cases were selected from the files of Parc de Salut Mar Biobank, Barcelona, Spain. IHC panel consisted of TTF-1 and p40 antibodies, and for older cases, p63 was available. Tissue was retrieved from paraffin blocks and processed for EM. The results of each technique were compared to the final diagnosis (gold standard), that was derived from the combination of light microscopy, IHC, EM, cytogenetics, molecular studies and data of the resection specimen if available.

Results: IHC concurred with the final diagnosis in 38 cases (79.2%) (Kappa=0.517). The identification of ADK by IHC had a sensitivity of 73%, specificity of 100%, positive predictive value (PPV) of 100% and negative predictive value (NPV) of 52.4%. EM results agreed with the final diagnosis in 35 cases (72.9%) (Kappa=0.471). For the diagnosis of ADK, IHC failed to recognize 10 cases (TTF1, P40 negative) and in all of them EM was conclusive, while in 10 cases with inconclusive EM, IHC gave the diagnosis. Thus, the values obtained for EM were identical to those of IHC: sensitivity 73%, specificity 100%, PPV 100% and NPV 52.4%. Combining results of IHC and EM, 47 cases (97.9%) were coincident with the final diagnosis (Kappa=0.943).

Conclusions: The results of this retrospective study support that EM can provide useful information in the diagnosis of NSCLC, mainly in recognizing poorly differentiated ADK, and that it has a particularly helpful role in cases in which IHC provides inconclusive results.

2025 Colour Deconvolution Is Superior to Hue-Saturation-Intensity in Digital Analysis of Atherosclerotic Plaques

Murad Alturkustani, J David Spence, Catherine Currie, Myra Cocker, Cathie Crukley, Martin J Yaffe, Jean-Claude Tardif, Cheemun Lum, Robert Beanlands, Robert Hammond, Ali Khan. King Abdulaziz University, Jeddah, Saudi Arabia; London Health Sciences Centre and Lawson Research Institute, London, Canada; University of Western Ontario, London, Canada; Robarts Research Institute, London, Canada; Ottawa Heart Institute, Ottawa, Canada; Montreal Heart Institute, Montreal, Canada; Sunnybrook Health Sciences Centre, Toronto, Canada.

Background: Digital analysis is becoming an essential tool in the quantitative analysis of immunohistochemically stained slides. The positive pixel algorithm is a widely applied method, built into the Aperio ImageScope software, and uses the Hue-Saturation-Intensity (HSI) colour-space to segment positively-stained pixels. An alternative approach, using colour deconvolution (CD), decomposes the image into separate channels for each stain using predefined colour samples. We sought to compare these two approaches

Design: This is a sub-study under the Canadian Atherosclerosis Imaging Network (CAIN) project of carotid plaque analysis that aims to improve the assessment of carotid atheromatous disease through studies that inform clinical imaging with gold-standard data (plaque pathology). One study component is to electronically annotate CD68 immunostained slides in atherosclerotic plaques for comparison with ultrasound, CT and PET data. Carotid endarterectomy specimens were sub-serially sectioned, stained, digitized and annotated manually and by electronic algorithms for CD68 immunostain. A set of 60 CD68-stained fields (from randomized subjects and slides) with corresponding segmentations (HSI, and CD) were examined in a blinded fashion by two neuropathologists (MA, RH). The neuropathologists were asked to choose the segmentation that best represented the labeling of positively stained tissue on the slide.

Results: The two observers found CD superior 94-100% of the time. It was also observed that the HSI algorithm did not identify the most heavily stained regions on the slide. This could be due to an innately poor ability to resolve differences in hue (H) when the intensity (I) is very low.

Conclusions: Colour deconvolution algorithms appear superior to those based on hue-saturation-intensity, especially in heavily-stained regions of the image.