

PULMONARY PATHOLOGY JOURNAL CLUB – MARCH 2023
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Discussion articles

Altorki N et al. Lobar or Sublobar Resection for Peripheral Stage IA Non-Small-Cell Lung Cancer. *N Engl J Med* 2023; 388(6):489-498.

Rusch VW. Initiating the Era of “Precision” Lung Cancer Surgery. *N Engl J Med* 2023;388(6):557-558. (editorial)

Purpose: Long awaited results of CALGB trial to prospectively test in a randomized fashion the efficacy of sublobar resection compared to lobectomy in relatively healthy patients with low stage peripheral NSCLC.

Methods:

- Multicenter (83 academic/community), international (US, Canada, Australia), noninferiority, phase 3 trial
 - relatively healthy patients (ECOG 0, 1, 2; no malignancy in last 3 years except non-melanoma skin cancer, superficial bladder cancer, cervical CIS; no chemo or rads for index lesion)
 - cT1a-b (solid component ≤ 2 cm) pN0 NSCLC
 - peripheral (center of tumor in outer third of lung and amenable to sublobar resection or lobectomy)
 - intraoperative confirmation (*frozen sections*) of NSCLC (if no preop dx) and node negative (unless previous mediastinoscopy or EBUS TBNA negative ≤ 6 weeks) disease
 - randomized 1:1 (stratified for tumor size, histologic type, smoking status) to either sublobar (wedge/segmentectomy) resection or lobectomy.
- Primary end point: disease free survival
- Secondary end points: overall survival, locoregional/systemic recurrence, pulmonary functions (FEV1 and FVC at 6 months).

Results:

- 1080 preregistered (125 surgeons) → 697 (64.5%) met eligibility criteria
 - Failure to randomize: benign disease (50%), higher stage disease (22.6%), malignancy \neq NSCLC (7.7%)
- 340 sublobar → 201 wedge (59.1%) + 129 (37.9%) segmentectomy
- 10 patients (4 sublobar + 6 lobectomy) died of treatment-related events within 90 days (excluded from recurrence calculations)

	Lobectomy (357)	95% CI	Sublobar (340)	95% CI	HR	90% CI
5-yr DFS	64.1%	58.5-69.0	63.6%	57.9-68.8	1.01	0.83-1.24
5-yr OS	78.9%	74.1-82.9	80.3%	75.5-84.3	0.95	0.71-1.27
5-yr RFS	71.2%	65.8-75.9	70.2%	64.6-75.1	1.05	0.8-1.39
Recurrence	103 (29.3%)		102 (30.4%)			
Locoregional	45 (13.4%)		35 (10.0%)			
Regional only	6 (1.8%)		9 (2.6%)			
Any distant	51 (15.2%)		59 (16.8%)			
LC deaths (101)	55		46			
Deaths other (93)	45		48			
FEV1 @ 6 mos	-6.0	-8.0 to -5.0	-4.0	-5.0 to -2.0		
FVC @ 6 mos	-5.0	-7.0 to -3.0	-3.0	-4.0 to -1.0		

Take-home message: In a highly selected group of patients with untreated peripheral clinical stage IA/pathologically confirmed N0 NSCLC, sublobar resection was noninferior to lobectomy with respect to DFS, OS, RFS, and pattern of recurrence. There were minor differences of limited clinical significance in physiological impact as measured by FEV1/FVC at 6 months. Combined with previously published Japanese study, they “*establish sublobar resection as the standard of care for a select group of patients with NSCLC.*” (Rusch editorial)

Suster DI et al. Epithelioid and Clear Cell Solitary Fibrous Tumors: Clinicopathologic, Immunohistochemical, and Molecular Genetic Study of 13 Cases. *Am J Surg Pathol* 2023;47:259–269.

Purpose: To report a histologically unique subset of solitary fibrous tumors occurring in multiple non-thoracic sites.

Methods:

- 13 cases (slides and blocks) from the Medical College of Wisconsin (12) and Johns Hopkins University Hospital (1).
- IHC for CK (AE1/3), vimentin, SMA, desmin, S100, CD31, CD34, bcl-2, MDM2, EMA, & STAT6; CD117 and DOG1 in selected cases.
- RNA-based fusion gene analysis using Archer FusionPlex (12) or JHU Sarcoma MDL Fusion Panel (1)
- 3 histologically similar cases with non-*STAT6* gene rearrangements were excluded.

Results:

- 8 F:5 M; mean age 63.1 ± 12.2 yrs (36-80)
- orbit (3), deep soft tissues of lower extremity (3), retroperitoneum (2), abdomen (2), and pelvis (1), superficial/subcutaneous soft tissues of mons pubis (1) and neck (1)
- mean diameter 9±6 cm (3.5-24.5 cm)
- “**sheets of large epithelioid cells** with a haphazard distribution and varying degrees of **collagenization**”
- “round to oval nuclei containing finely dispersed chromatin and abundantly **clear to eosinophilic cytoplasm**”
- “**low to intermediate-grade** histology with **minimal to mild cytologic atypia**, absence of nuclear pleomorphism, **<3 mitoses per 10 HPFs** [≤ 3 in 6 cases; > 3 in 7 cases] and **absence of necrosis**.”
- “striking **nesting** appearance” in 6; “superficially resembling the **whorling pattern of meningiomas**” in 3 (all orbital); **hemangiopericytoma-like** vascular pattern in 7; conventional SFT in 4
- IHC (13): CD34+ (11); STAT6+ (10); CD34–/STAT6– (1); bcl-2 (12); negative CK, EMA, SMA, desmin, S100, MDM2.
- *NAB2::STAT6* gene fusion in all 13; 4 different transcript variants

Demicco risk	lost to f/u	A&W	local recurrence	 mets	dead of disease
low (6)	2	3	1 (1 yr)	1 (2 yrs)	1 (8 yrs)
intermediate (5)		3	1 (4 yrs)		
high (2)			1 (4 yrs)	1 (8 mos)	1 (1 yr)

Take-home message: Epithelioid and clear cell SFT is diagnostically challenging and has been previously described in the mediastinum (Marchevsky 2003) and pleura (Yan 2008). Meningioma a potential differential diagnosis in orbital tumors. CD34 and STAT6 IHC the right next step in a keratin-negative tumor but can be negative making molecular testing an important option.

Hsieh MS et al. Constant p.L424H Mutation in GTF2I in Micronodular Thymomas With Lymphoid Stroma: Evidence Supporting Close Relationship With Type A and AB Thymomas. *Mod Pathol* 2023;36(2).

Purpose: *GTF2I* (codon L424H) is a thymoma-specific oncogene that is enriched in type A & AB thymomas; rare in type B thymomas. Anecdotal reports, including TCGA (whole exome), identified *GTF2I* p.L424H mutations in 4 of 6 micronodular thymomas with lymphoid stroma (MTLS). The authors used a highly sensitive NGS assay and manual microdissection to compare and contrast molecular alterations in MTLS and micronodular thymic carcinoma with lymphoid stroma (MTCLS).

Methods:

- study group (reviewed by 3 pathologist authors): 12 MTLS (2 with type A components) and 2 MTCLS characterized by being “almost indistinguishable” from MTLS at low power but “cytologic atypia, large vesicular nuclei, conspicuous nucleoli, and brisk mitotic activity” at high power.
- manually microdissected micronodular thymoma, type A thymoma, and micronodular thymic carcinoma components
- “a highly sensitive next-generation sequencing (NGS) assay using the molecular barcoding Ion AmpliSeq HD technology” (“utilizes dual molecular barcodes to detect rare somatic variants”) was applied to include commonly mutated genes in thymoma: *GTF2I*, *HRAS*, *NRAS*, *KRAS*, *TP53*.
 - primers designed to enable “unambiguous discrimination of reads from *GTF2I* and the pseudogenes [*GTF2IP1*, *GTF2IP4*]”
 - excluded variant allele frequencies (VAF) < 2% “to minimize false-positive calls”
- all cases also interrogated using Sanger sequencing (the platform that reported p.L424H *GTF2I* mutations in 3 of 4 cases)

Results:

	MTLS (10)	MTLS + type A (2)	MTCLS (2)
F:M	7:3	2:0	1:1
Age (years)	mean 68.0 ± 8.5 (53-80)	74, 72	73, 49
Tumor size (cms)	mean 5.6 ± 4.4 (2.0-16.5)	10, 3.8	5, 2.4
Masaoka-Koga stage	I – 4 IIA – 6	I, IIA	IVA, I
Follow-up (mos)	A&W (mean 44 ± 42.6; range 3-124)	A&W (110, 4)	A&W (50, 30)
NGS			
<i>GTF2I</i> p.L424H mut	10 (100%)	2 + 2	0
VAF (mean ± STD)	10.4% ± 5.2% (4.9%-22.9%)	17.6, 17.0 + 21.4, 31.5	
<i>HRAS</i>	1*	1* + 1	
<i>KRAS</i>	1	1 (MTLS = * <i>HRAS</i> + <i>KRAS</i>)	
<i>NRAS</i>	0	0	0
<i>TP53</i>	1 (* <i>HRAS</i> + <i>TP53</i>)	0	2
Sanger seq			
<i>GTF2I</i> p.L424H mut	1 (10%)	2	0

Take-home message: Recurrent p.L424H mutations in *GTF2I* are common in MTLS, type A (100%), and type AB (70%) thymomas consistent with Suster and Moran’s 1999 proposal that MTLS is a subset of the latter. *GTF2I* mutations do not occur in MTCLS which are more likely to harbor TP53 mutations. Sanger sequencing is an insensitive method for detecting p.L424H *GTF2I* mutations in thymoma given relatively low VAF.

Vehar SJ et al. Smoking-Related Interstitial Fibrosis (SRIF) in Patients Presenting with Diffuse Parenchymal Lung Disease. *Am J Clin Pathol* 2023;159:146-157.

Purpose: Smoking-related interstitial fibrosis (SRIF) is the term coined by Katzenstein et al to describe a pattern of fibrosis discovered as an “incidental” finding in lobectomies from cigarette smokers. It’s co-existence with RB and DIP-like changes implied that in a subset of patients it likely accounted for a syndrome of mild restrictive lung disease as reported by Yousem who proposed the term *RB-ILD with fibrosis* (Mod Pathol 2006). These authors summarize their experience with SRIF in patients with clinical/radiological evidence of diffuse parenchymal lung disease (DPLD).

Methods:

- Retrospective cohort of patients reviewed at Cleveland Clinic’s weekly DPLD conference (2013-2022) who, 1) were suspected of having DPLD, 2) had SRIF on surgical lung biopsy (SLB), and 3) had slides and chest CT scans available for “focused multidisciplinary re-review”.

Results:

- 6 patients met inclusion criteria
 - 5 F:1 M; mean age 47 yrs (42-57); 5 current/1 former (3 mos) smokers (15-55 pack years; median 27.5)
 - all were symptomatic with mMRC dyspnea scores of 1 (SOB when hurrying on the level or walking up a slight hill), 2 (walks slower than people of same age on the level because of breathlessness or has to stop to catch breath when walking at their own pace on the level), 3 (stops for breath after walking ≈ 100 m or after a few minutes on the level) and 4 (too breathless to leave the house, or breathless when dressing or undressing) in two, one, one, and two patients, respectively
 - mild obstructive ventilatory defect (COPD) in 1 and 2 patients by ATS and GOLD criteria, respectively; 2 (of 5) had restrictive defect by TLC (59%, 74%); median DLco 53% (33%-68%)
 - follow-up at 8-116 mos (median 37.5), including repeat imaging: persistent symptoms, supplemental O2 in 3; stable (5) to improved/resolved (1)
- bilateral GGOs in diffuse bilateral (5) (upper [2] and lower [2] lobe predominant) distributions. Bronchiolocentric in 1. Mediastinal/hilar adenopathy in 5. Emphysema in 2.
- pathology as previously described: subpleural and peribronchiolar, in 1 case “mimicking the histologic appearance of NSIP” in UL; “mild pathologic emphysema” and “Pigmented macrophages (‘smokers’ macrophages’)” in all of them (with diatribe in discussion re why this is better terminology than “respiratory bronchiolitis”); lymphoid aggregates w/out germinal centers common; focal honeycombing in 1 (“misdiagnosed as usual interstitial pneumonia [UIP] at another institution”).

Take-home message: SRIF usually not associated with HRCT predictors of fibrosis (ie, reticulation, traction bronchiectasis/’iolectasis, honeycombing). Fibrotic NSIP and UIP the main differential diagnosis. Discussion reviews challenges in harmonizing language around smoking-related interstitial lung diseases, arguing that SRIF better than RBILD and DIP while also concluding that “*Whether the distinction between the overlapping categories of RBILD, DIP, and SRIF is clinically significant is worthy of further study.*”

Articles for notation

Neoplastic

Yun JK et al. Clinical Impact of Histologic Type on Survival and Recurrence in Patients with Surgically Resected Stage II and III Non-Small Cell Lung Cancer. *Lung Cancer* 2023 Feb;176:24-30.

Purpose: Explore impact of histologic classification on overall survival (OS)/freedom from recurrence (FFR) in a cohort of 2,155 patients with stage II (51.8%) and III (48.2%) squamous cell carcinoma (SqCC) (33.4%) or adenocarcinoma (ADC) (66.6%) following complete surgical resection at Asan Medical Center in South Korea between JAN2008 and DEC2018. Excluded patients with concurrent malignancies, neoadjuvant therapy, sublobar or incomplete resection.

Results: Statistically significant ($p < 0.001$) differences between cohorts indicated that patients with SqCC were older and more likely to be male, ever smokers, and have ≥ 1 comorbidities, poorer FEV1/DLco, larger tumors, more extensive resections (more bilobectomies, pneumonectomies, and sleeve resections), more frequent thoracotomy, and less frequent adjuvant chemotherapy for stage III disease. *EGFR* testing in 434 (55.6%) stage II and 567 (76.3%) stage III ADC patients; *EGFR* mutations detected in 48.4% stage II and 33.9% stage III ADC patients (39.3% overall). *ALK* testing in 158 (22.8%) stage II and 264 (35.6%) stage III ADC patients; *ALK* rearrangements in 12.0% stage II and 15.9% stage III ADC patients (14.5% overall). Targeted therapy in 86 (12.4%) stage II and 252 (33.9%) ADC patients with *EGFR/ALK* alterations, “mostly after cancer recurrence”.

Take-home message: Patients with SqCC had better FFR (stage II & III – $p < 0.001$) with equivalent OS at 5 years driven in large part by higher rates of non-cancer related deaths in patients with SqCC (stage II – $p < 0.001$; stage III – $p = 0.039$) “*due to the differences in baseline characteristics*”. Time from lung cancer recurrence to death was shorter in SqCC patients (median: stage II, 13 vs 37 mos – $p < 0.001$; stage III, 11 vs 26 mos – $p < 0.001$) and was “*closely related to the different rates of targeted therapy*”.

Wolf JL et al. The Value of Prognostic and Predictive Parameters in Early-Stage Lung Adenocarcinomas: A Comparison Between Biopsies and Resections. *Lung Cancer* 2023;176:112-120.

Purpose: To test differences in prognostic/predictive parameters for lung adenocarcinomas (LUAD) assessed on biopsies (JAN2009-DEC2018: 7,226 lung biopsies → 367 LUAD → 187 study patients) versus resections (123 at reporting institution ← 126 lobectomy/wedge ← 187 study patients) in retrospective case series of stage I (166 – 87.4%) and II (24 – 12.6%) LUAD patients (stage subsets total 190?) treated at a single medical center in the Netherlands.

Methods and Results: Tested histologic parameters were WHO growth pattern, nuclear grade, fibrosis (*ie*, desmoplastic stromal reaction), inflammation, and available results for molecular testing. Univariate analysis identified age, sex, T stage, N stage, clinical stage, growth pattern, nuclear grade, driver mutations, and *TP53* mutations as significant factors in predicting worse DFS/OS. Multivariate analysis identified T2 tumor, N1 disease, presence of *KRAS* mutation, and absence of a driver mutation as significant predictors of worse DFS/OS. ROS analysis tested various combinations of prognostic/predictive parameters and showed better performance for resection specimens compared to biopsies.

Take-home message: High-grade histologic patterns (micropapillary, solid) in both biopsies and resections predicted for worse survival, with greater separation of low-grade and intermediate-grade groups for resections attributable to the frequency of “upgrading” in matched pairs (18 lepidic → 14 acinar, 3 micropapillary, 1 solid and papillary; 2 acinar → solid) (overall concordance for dominant growth pattern 73.6%)

Terada K et al. Clinicopathological Features of Cytokeratin 5-Positive Pulmonary Adenocarcinoma. *Histopathology* 2023;82:439-453.

Purpose: Japanese cohort study of CK5-positive adenocarcinomas (ADC) combining the results of 220 resected (\leq IIIA) tumors (\geq 10% of tumor cells) for “studying the detailed morphologic characteristics” and a 337 specimen TMA (any CK5-positive tumor cells) constructed from resected tumors “for investigating the associations of CK5 expression with other protein expressions, genetic and prognostic factors”.

Results: CK5-positive ADC, defined differently in these two cohorts, accounted for 91 (16.3%) of cases and was statistically more likely ($p < 0.001$) to occur in ever smokers, be greater than 2.0 cm in maximum diameter, have stage II-IV disease, node mets, visceral pleural and vascular invasion, mucinous differentiation, a high-grade histologic component, and STAS. As assessed on TMA they were p40 negative and *more likely* ($p < 0.001$) to be positive for MUC5B and HNF4alpha. TTF1 co-expressed in CK5-positive subset of tumor cells. No CK5 observed in 9 AISs and 39 MIAs and in only 1 (of 28) lepidic predominant adenocarcinomas. They were *less likely* ($p < 0.001$) to be *EGFR* mutated (23.6% vs 54.6%) and *more likely* ($p < 0.001$) to have *KRAS* mutations (23.6% vs 9.6%) and *ALK* rearrangements (7.7% vs 0.9%).

Take-home message: CK5 staining occurs in a subset of lung ADC that tend to be mucinous, are more likely to have high grade histologic components, more likely to be *KRAS* and less likely to be *EGFR* mutated and affiliated with lower DFS and OS.

Blaauwgeers H et al. Prognostic Value of Proliferation, PD-L1 and Nuclear Size in Patients with Superior Sulcus Tumours Treated with Chemoradiotherapy and Surgery. *J Clin Pathol* 2023;76:111–115.

Purpose: To examine whether changes in nuclear size, and expression of MIB-1 and PD-L1 are prognostically relevant in a retrospective case series of 33 consecutive patients with residual tumor (ADC 19, SqCC 12, NSCLC NOS 2) following neoadjuvant chemoradiation and resection for superior sulcus tumors.

Take-home message: Lower Ki67 indices ($< 20\%$) predicted for significantly better DFS ($p < 0.001$) and OS ($p = 0.016$). Negative ($< 1\%$) PD-L1 predicted for better OS ($p = 0.011$) but not DFS ($p=0.13$). Nuclear size did not differ between pre-treatment biopsies and post-treatment resections and was not significantly associated with DFS/OS (whew!).

Avasarala SK et al. Rapid On-Site Evaluation Practice Variability Appraisal (ROSE PETAL) Survey. *Cancer Cytopathol* 2023;131:90-99.

Purpose: To survey the two largest groups of interventional pulmonologists and advanced diagnostic bronchoscopists in North America (American Association for Bronchology and Interventional Pulmonology and the Society for Advanced Bronchoscopy) regarding their experience and perceptions of utilization patterns, barriers, perceived benefits, limitations, and result disclosure patterns of ROSE for EBUS TBNA and any peripheral bronchoscopy with radial probe ultrasound (did not include percutaneous needle biopsy).

Results: A large majority (80%) of 137 respondents (8% response rate) to a survey open for 24 consecutive days in February and March 2022 self-identified as trained interventional pulmonologists. Just over half (52%) were in academic medical centers. Nearly all (88%) reported use of ROSE at their institution for which the primary readers (person reporting results at the time of the procedure) were attending cytopathologists in 55%.

Take-home message:

- 1) Time constraints (28%), availability of cytology (22%), and scheduling conflicts (20%) were the most frequently reported barriers to ROSE use.
- 2) Endobronchial ultrasound transbronchial needle aspiration (85%) and nonrobotic peripheral bronchoscopy (65%) were the most reported procedures that used ROSE.
- 3) 38 (28%) respondents reported they believed ROSE was $\geq 90\%$ concordant with final cytology results.
- 4) There was heterogeneity regarding discussion of ROSE results with the patient or their caregiver in the immediate post-procedure setting: **YES** – always (40, 33%), sometimes (32, 26%), rarely (18, 15%); **NO** (31, 26%).

Wang Z et al. Sputum Cell-Free DNA for Detection of Alterations of Multiple Driver Genes in Lung Adenocarcinoma. *Cancer Cytopathol* 2023;131:110-116.

Purpose: Cell free DNA (cfDNA) from sputum has been demonstrated to be useful for identifying EGFR mutations in patients with lung adenocarcinoma (LAC). The same group of Chinese (Beijing) collaborators sought to demonstrate value of sputum cfDNA for other driver mutations using a 10-gene NGS panel (*EGFR*, *KRAS*, *BRAF*, *NRAS*, *HER2*, *PIK3CA*, *ALK*, *ROS1*, *RET*, and *MET*) to analyze sputum cfDNA and paired tissue samples from a consecutive series of 83 stage I-IIIa (14) and IIIB-IV (69) LAC patients. Ten non-tumor negative control sputum cfDNA samples were also tested. Sputum samples examined via ThinPrep were malignant in 51 (61%) and satisfactory but negative for malignant cells in 32 (39%).

Take-home message: It works!! Specificity 100% and overall sensitivity 81.3% (95% CI, 69.2%-89.5%); sensitivity for SNV/Indels were higher at 83.9% (95% CI, 71.1%, 91.9%) compared to fusions at 62.5% (95% CI, 25.9%, 89.8%). Sensitivity highest in patients with stage IIIB-IV disease and positive cytology, and lowest in patients with stage I-IIIa disease and negative cytology.

De Miguel-Perez D et al. Baseline Extracellular Vesicle TGF- β is a Predictive Biomarker for Response to Immune Checkpoint Inhibitors and Survival in Non-Small Cell Lung Cancer. *Cancer*. 2023;129:521–530.

Purpose: Transforming growth factor- β (TGF- β) is an immunosuppressive cytokine found free in circulation in the blood and packaged into extracellular vesicles (EVs). It plays a crucial role in tumor immune escape, treatment resistance, and metastasis. The authors set out to evaluate the predictive value of circulating and EV TGF- β in a retrospective cohort of 33 patients with advanced stage non-small-cell lung cancer treated with immune checkpoint inhibitors (ICIs) pembrolizumab/nivolumab at a medical center in Messina, Italy. Plasma was obtained from blood samples drawn before, and 9 \pm 1 weeks after, ICI therapy was initiated.

Take-home message: In this pilot study, pre-treatment EV TGF- β outperformed circulating TGF- β and tissue PD-L1 in predicting treatment response (AUC = 70.4%, compared to 55.9% and 62.6%, respectively) and survival impact of ICI therapy. Nonresponders showed higher pre-treatment levels of EV TGF- β than responders ($p = .047$). Patients with low pre-treatment EV TGF- β experienced longer PFS (HR, 0.45; $p = .046$) and OS (HR, 0.35; $p = .026$).

Esposito Abate R et al. External Quality Assessment (EQ) for Tumor Mutational Burden: Results of an International IQN Path Feasibility Pilot Scheme. *Virchows Archiv* 2023;482:347-355.

[Correction to: External Quality Assessment (EQ) for Tumor Mutational Burden: Results of an International IQN Path Feasibility Pilot Scheme. *Virchows Archiv* 2023;482:357.]

Purpose: TMB was approved by the Food and Drug Administration (FDA) as an agnostic biomarker for the treatment with pembrolizumab of adult and pediatric patients with unresectable or metastatic TMB high (≥ 10 mut/Mb) solid tumors. The eligible method to assess this potential biomarker is whole exome sequencing

(WES), but the costs, the turnaround time, and the necessary minimum coverage make this approach largely unfeasible in clinical practice. TMB quantification with targeted next generation sequencing (NGS) panels has been shown to correlate with WES-derived TMB and to associate with ICI response, making the clinical assessment of TMB practically achievable. FoundationOne CDx (F1CDx) and MSK-Impact are the only two targeted sequencing panels currently approved by the US Food and Drug Administration to assess TMB. The International Quality Network of Pathology (IQN Path), a network of quality assessment associations with an interest in cancer biomarker testing, organized a pilot external quality assessment (EQA) scheme for TMB testing with the collaboration of different academic partners to validate the materials and procedures for EQA of this complex biomarker in 23 of 29 originally enrolled laboratories from 12 different countries.

Take-home message: As anticipated, the use of different technologies for TMB testing led to significant variability in the reported TMB values with reasonable reproducibility across labs using common platforms. This pilot study demonstrated a reproducible methodology for external quality assessment of TMB testing that included validation of control material.

Cheung AHK et al. Interpretation of Lung Cancer Plasma *EGFR* Mutation Tests in the Clinical Setting: An Update with Meta-Analysis. *Am J Clin Pathol* 2023;159:181-191.

Purpose: Plasma *EGFR* mutation testing is useful for determining patient response to therapy, detecting the emergence of targeted therapy resistance, monitoring disease status, gauging patient eligibility for targeted therapy, and demonstrating the clonality relationship between a new lesion and a known *EGFR*-positive tumor. Examined how likely it is that a patient with given clinical parameters will have a false-negative plasma *EGFR* mutation test result.

Methods & Results: Combined

- 1) literature review (*ie*, meta-analysis of publications analyzing cell free circulating tumor DNA [ctDNA] + ≥ 1 clinical or imaging characteristic, JAN2001-DEC2021 \rightarrow 14 studies, all Asian, of 2,546 patients) with a
- 2) single institution retrospective observational cohort study of 170 (168 patients) plasma *EGFR* mutation testing from 2015 through 2021 at a “tertiary referral and university teaching hospital” (State Key Laboratory of Translational Oncology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong) with paired *EGFR* testing of tissue or cytology specimens ($n = 75$). “False negative” = negative result in patient for whom “at least 1 *EGFR*-mutated lesion was reasonably likely to be present at the time of plasma *EGFR* testing”. 72 (42.4%) plasma *EGFR* mutations tests were positive. 15 of 98 negative tests were judged to be false negative results. Comparison of false negatives to true positives had comparable clinical courses. Fewer patients with false-negatives had metastases or distant mets; patients with true-positives had more extensive organ involvement with significantly higher numbers of suspicious nodes on imaging studies.

Take-home message: The sensitivity of plasma *EGFR* testing is highest in patients with stage IV disease, and more heterogeneous in patients with stage I-III disease.

Churg A and Naso JR. Hypothesis: HEG1 and Claudin-4 Staining Will Allow a Diagnosis of Epithelioid and Biphasic Mesothelioma Versus Non-Small-Cell Lung Carcinoma with Only Two Stains in Most Cases. *Histopathology* 2023;82:385-392.

Purpose: In 100 consecutive consultation cases reviewed by Dr. Churg, 93 had more than 4 stains (range 3-30, mean 9.6 ± 4 , median 9.0) leading to greater likelihood of a discordant signal. This study reviewed published literature regarding the efficacy of membranous staining for *HEG homologue 1* (HEG1) and claudin-4 in distinguishing NSCLC from epithelioid/biphasic mesothelioma.

Results: HEG1 testing in 4 different laboratories was both sensitive (91%; 393 of 434) and specific (99.7%; 1 of 360) for epithelioid/biphasic mesothelioma compared to NSCLC, with lower specificity in serous and thyroid carcinomas. Sensitivity “modest and staining sometimes difficult to interpret” for sarcomatoid mesothelioma. Claudin-4 testing in 7 different laboratories was equally sensitive (93%; 469 of 502) and specific (98.9%; 5 of 463) for NSCLC compared to epithelioid/biphasic mesothelioma.

Take-home message: Immunohistochemistry applied in a clinically, radiologically, and histopathologically appropriate context using a combination of HEG1 (SKM9-2) and claudin-4 will solve the problem of separating metastatic NSCLC from epithelioid/biphasic mesothelioma in the vast majority of cases.

HEG1	Claudin-4	Interpretation
+	—	mesothelioma
—	+	NSCLC
+	+	carcinoma (serous?)
—	—	indeterminate

Non-Neoplastic

Wismans VL et al. Increase of Mast Cells in COVID-19 Pneumonia May Contribute to Pulmonary Fibrosis and Thrombosis. *Histopathology* 2023;82:407-419.

Purpose: The authors note that AFOP, OP and DAD are not limited to viral pneumonias and draw analogy to chronic lung allograft dysfunction (CLAD) in lung transplant patients (?). They set out to understand “whether autoimmune-related inflammatory reaction plays an important role in COVID-19” using a combination of gene expression profiling and immunofluorescence for tryptase and chymase in autopsy lungs from 18 COVID-19 patients and 9 influenza patients.

Take-home message: Several genes involved in fibrosis and thrombosis are up-regulated in COVID-19 and mast cells are significantly increased compared to influenza.

Bodmer JL et al. Obstructive Bronchial Fibrin Case Formation in COVID-19 Severe Respiratory Failure. *M J Respir Crit Care Med* 2023;207(3):349-350.

Purpose: To report the finding of plastic bronchitis in autopsy lungs of 17 year-old female who died of COVID-19. She also had “numerous acute and focally organizing thrombi in the pulmonary artery distribution”.

Take-home message: So far as I know the only description of plastic bronchitis at autopsy in a patient with COVID-19. Hard to know what, if anything, that means.

Smoot M et al. A Woman With Progressive Dyspnea and Seronegative Rheumatoid Arthritis. *CHEST* 2023 163(2):e63-e67.

Purpose: To showcase the term “hypersensitivity pneumonia with autoimmune features” (HPAF) in a patient with RA who suffered from HP.

Take-home message: xPAF is officially out of control.

Thang ND et al. A 44-Year-Old Man with Chronic Lower Chest Pain. *CHEST* 2023;163(2):e69-e72.

Purpose: Beautifully illustrated bronchogenic cyst arising from the diaphragm in a symptomatic adult.