Pulmonary Journal Club April 2024 (Articles from March 2024)

Presented by

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Articles for Discussion

Hong TH et al. Clinical significance of the proposed pathologic criteria for invasion by the International Association for the Study of Lung Cancer in resected nonmucinous lung adenocarcinoma. J Thorac Oncol 2024;19: 425-33

Editorial by Borczuk A. J Thorac Oncol 2024;19:360-362

Background/Purpose:

- 8th TNM pT staging for lung cancer requires accurate assessment of invasive size (excluding the area with lepidic pattern)
- Better prognostigation for early stage subsets
- Reproducibility is the problem
- IASLC Pathology Panel working group focused on this problem of reproducibility
- Interobserver agreement was not randomly varied but clustered into two groups
- Invasion Working Group formed by IASLC Pathology Committee reappraised the criteria for assessing tumor invasion by Delphi process and the group identified the following:
    - Iatrogenic alveolar collapse as a prime source of pathologic overstaging
    - Extensive epithelial proliferation as a source of pathologic understaging
    - Supplemental Figure 1 & 2
- Clinical usefulness of the proposed criteria has not been clarified
- Authors aimed to evaluate the clinical significance of the IASLC-proposed invasion criteria using a registry of part-solid nonmucinous lung adenocarcinoma
    - To see whether applying the proposed IASLC criteria would result in a more precise pT staging, ultimately leading to improved prognostication
    - To evaluate association between the proposed criteria and radiologic/ pathologic risk factors that serve as surrogates for tumor invasiveness

Materials and Methods

- Retrospective cohort: using the registry for thoracic cancer surgery data at Samsung Medical Center, Seoul Korea
    - 1,487 patients in cT1-3(according to 8th)N0 lung ADC with radiologic part-solid (but not pure GGO or pure solid) appearance who underwent surgical resection with curative intent from 1/2017 – 12/2019

    - Exclusion: neoadjuvant (n=24), invasive mucinous ADC or other variants of ADC (n=108)
    - 1,295 cases divided into two groups and compared the performance: Revivsed pT category and Conventional pT category, with and without IASLC criteria applied, respectively
- Histologic evaluation: perfusion fixed; 3.64 tissue blocks/ case (1.49/tumor cm); 2 thoracic pathologists; original pT and revised pT by IASLC criteria; discrepant cases with deliberate consideration; doubtful cases being upgraded on the basis of the IASLC pathology committee's flowchart (supplemental Figure 2)
- Outcomes:
    - performance of revised pT stage, compared with the orginal pT stage, in predicting RFS and proof of invasion status (i.e. the presence of LN met or recurrence) *although the absence of recurrence or LN met does not necessarily indicate the absence of invasion*
    - secondary outcome: correlation of the pT stage with the radiologic surrogate of tumor invasiveness, such as SUVmax, radiologic consolidation-to-tumor ratio, pathologic risk factors (LVI, necrosis, high-grade histologic pattern, i.e. micropapillary, solid or complex glands)
- Statistical analysis:
    - Continuous variables: median, IQR; Categorical variables: frequencies and %
    - Appropriate statistics
    - Predictive performance of pT stage using the AUC

Results:

- Patients characteristics in Table 1
- # of patients according to original and revised T stage (Figure 2)
- Figures 3 and 4 show performance of each system for various parameters

Take Home Points/Discussion: The proposed IASLC criteria did offer better alignment with clinicopathologic risk factors and improve prognostication.

Yang SR et al. Microsatellite instability and mismatch repair deficiency define a distinct subset of lung cancers characterized by smoking exposure, high tumor mutational burden, and recurrent somatic *MLH1* inactivation. J Thorac Oncol 2024;19:409-424

Editorial by Cani M, et al. J Thorac Oncol 2024;19:363-365

Background/Purpose

- The clinical and genomic landscape of MSI-H and MMR-D in lung cancer remains poorly defined in lung cancers compared to other organs
- It is also unclear whether MSI-H and MMR-D interact with other immune markers such as PD-L1, TMB, and TILs

- To address these issues, they prospectively evaluated MSI and MMR-D in 5,171 NSCLC and 316 SCLC patients by using targeted NGS (MSK-IMPACT and MSK ACCESS)

Materials and Methods

- Cases: Retrospective search for tissue and plasma samples with de novo NSCLC and SCLC genotyped by MSK-IMPACT and MSK-ACCESS during 2014-2020
    - MSK-IMPACT testing: on tumor tissue and matched blood samples (normal)
    - MSK-ACCESS testing: on cell free DNA from plasma (tumor) and matched buffy coat (normal)
- To confirm MSI-H/MMR-D status, Idylla MSI and MMR IHC (for MSK-IMPACT cases only) were performed
- Other tests: PD-L1 IHC, MLH1 promoter hypermethylation analysis, whole exome sequencing, germline analysis (in select cases), TCGA analysis for comparison
- Clinicopathologic data analysis from medical records
- Data analysis: Fisher's exact test for comparison of proportions; Mann-Whitney U test for medians; Benjamini-Hochberg method for comparing mutations

Results

- MSI-H/MMR-D in 21 NSCLC (0.41%) and 6 SCLC (1.9%) patients
- All were smokers including 11 adenoca patients
- Compared to MSS cases: NSCLC with MSI-H/MMR-D with higher TMB (37.4 vs. 8.5 muts/Mb $p < 0.0001$), MMR mutational signature (43% vs. 0%), somatic biallelic alterations in *MLH1* (52% vs. 0%); SCLC had similar features and also with *MLH1* promotor hypermethylation
- Advanced MSI-H lung cancers treated with ICIs: durable clinical benefit in 3 of 8 NSCLC (*STK11, KEAP1, JAK1* mutations in nonrespondors) and 2 of 2 SCLC patients

Take Home Points/Discussion: NSI-H and MMR-D define a rare subset of lung cancer associated with smoking exposure, high TMB, and somatic *MLH1* inactivation. The broad range of responses to ICI thearpy suggests an influence by co-mutational landscapes

Febres-Aldana CA et al. Comparison of immunohistochemistry, next-generation sequencing and fluorescence in situ hybridization for detection of MTAP loss in pleural mesothelioma. Mod Pathol 2024;37: 100420

Background/Purpose

- Loss of MTAP expression by IHC is a well accepted surrogate for 9p21 deletions involving *MTAP/CDKN2A* genes
- Interpretation of MTAP IHC can be challenging in many cases
- Variations in Ab performance may impact interpretation
- Objectives: To compare the performance of MTAP monoclonal antibodies EPR6893 (Abcam) and 1813 (Novus Biologicals).

Materials and Methods

- 56 DPM; 47 epithelioid, 7 biphasic, 2 sarcomatoid
- Profiled by targeted NGS, 9p21 copy number analysis by FACETS (Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing)
- CDKN2A FISH for in discrepant cases between IHC and NGS finding

Results

- 19 of 56 (34%) DPM cases tested with mAb EPR6893 showed equivocal interpretation
- Equivocal interpretation with mAb EPR6893 was due to weak "blush" cytoplasmic stainng (n=10), poor internal control (n=6) and heterogenous staining (n=3)
- 17 of 56 (30%) showed retained MTAP and 20 of 56 (36%) showed loss by mAb EPR6893
- 21 of 56 (37%) showed retained MTAP and 35 of 56 (63%) showed loss by mAb 1813
- No case with equivocal staining with mAb 1813; stronger immunoreactivity overall, reduced nonspecific "blush" background staining (often seen with mAb EPR6893), and also better positive internal control
- Subclonal loss of MTAP expression with mAb 1813 was seen in epithelioid DPM, that was interpreted as equivocal with mAb EPR6893
- Also, mAb 1813 showed excellent performance using combined NGS (FACETS) and *CDKN2A* FISH results with homozygous deletion as the gold standard: sensitivty 96%, specificity 86%, accuracy 93% (Figure 4)
- Discrepancies between NGS-based copy number calls and MTAP expression by mAb 1813 IHC was mainly in samples with low tumor purity ($\leq$ 20%)
- FISH confirmed homozygous deletion in 7 of 8 discordant low-purity cases, supporting the MTAP loss result by IHC using mAb 1813 clone

Take Home Points/Discussion

According to this study mAb 1813 (NBP2-75730; Novus Biologicals) is better! *MCR uses clone 2G4 by Abnova company*

Linea M et al. Multicenter evaluation of an automated multiplex, RNA-based molecular assay for detection of ALK, ROS1, RET fusions and MET exon 14 skipping in NSCLC. Virch Arch 2024

Background/Purpose

- Timely therapeutic decision making is essential in the tx of advanced NSCLC
- Increased number of biomarkers are tested on very sparse material with multiple testing technologies, which led to undesired prolonged time to tx
- This multicenter study investigated the performance of Idylla^{TM} GeneFusion Assay in a real-life clinical setting involving 12 clinical centers across Europe

Materials and Methods

- 12 clinical centers (Belgium, Czech, Denmark, 3 in France, 2 in Germany, Italy, 2 in Spain and Switzland)
- Multicenter observational non-interventional retrospective study to assess the mutational status in 326 archival histologically proven advanced NSCLC (stage IV) FFPE (tissue or cytological) samples with the Idylla™ GeneFusion Assay
- The results were compared with routine reference methods including FISH, IHC, RT-PCR and NGS that had been performed previously for *ALK, ROS1, RET* fusions and/or *MET* exon 14 skipping
- Using with same block at the same clinical centers with the Idylla™ GeneFusion Assay
- Exclusion: stained sample, non-FFPE samples, decalcified samples, samples older than 9 years (or 3 years if compared to FISH)
- Tissue sections(s) were placed in the lysis chamber of a new cartridge, then loaded onto the Idylla™ instrument (taking about 2 min of hands on time)
- The technique can be used on demand without batching, the results available within 3 h

Results

- Two of 326 Idylla™ GeneFusion Assays resulted in an error; the final analysis set included 324 samples (150 bx, 144 resection, 28 cytological, 2 unknown) (Table 2)
- Idylla™ GeneFusion Assay generated 323 valid overall test results (Table 3); one invalid test due to RNA degradation; i.e. validity of the Assay 99.1% (323/326)
- Idylla™ GeneFusion Assay also measured expression imbalance of *ALK, ROS1,* and *RET* genes in addition to particular fusion events
- Agreement between Idylla™ GeneFusion Assay and the routine reference methods (including the expression imbalance results): 312 (96.3%) *ALK* fusion, 315 (97.2%) *ROS1* fusion, 321 (99.1%) *RET* fusion, and 319 (98.5%) *MET* exon 14 skipping
- Analysis of discordant results by the third method (Table 4); it was considered discordant if a third method could not be performed or its result was inconclusive
- Final agreement of Idylla™ GeneFusion Assay (including expression imbalance) with reference method results: 98.8% for *ALK* fusion, 99.4% for *ROS1* fusion, 99.4% for *RET* fusion and 98.8% for *MET* exon 14 skipping

Take Home Points/Discussion

Idylla™ GeneFusion Assay is a promising tool for rapid detection of *ALK, ROS1, RET,* or *MET* exon 14 alterations in NSCLC

Suster D et al. Non-small cell lung carcinoma with clear cell features and FGFR3::TACC3 gene rearrangement. Am J Surg Pathol 2024;48:284-291

Background/Purpose

- Conventional lung cancer with clear cell morphology is regarded as a rare morphologic variant of NSCLC that is of interest mainly for ddx with other clear cell tumors of the lung but not as distinctive and discrete entity
- Little is known about their biologic behavior; likely corresponds to that of other stage matched ADC or sqcc
- A previous study by Yousem showed *KRAS* mutation as most common alteration by PCR and FISH
- Authors encountered 7 cases of lung ca with clear cell features having *FGFR* alterations by NGS and described the clinicopathologic immunohistochemical and molecular genetic features of these tumors and the potential role of this findings for targeted therapy

Materials and Methods

- Retrospective review of clear cell tumors of the lung from the surgical pathology files from one institution
- Inclusion criteria: primary lung cancer by clinical hx and f/u, epithelial phenotype (keratin positivity), >50% clear cell change in the tumors cells (*not entirely clear how many cases were found in their initial search…)*
- IHC: AE1/AE3, CK7, CK5/6, TTF1 (8G7G3/1), p40, Napsin A, MIB1
- Molecular testing: fusion and variant analysis using 2 different NGS assays
    - RNA-seq NGS using a custom 94 gene Archer FusionPlex panel that includes gene-specific primers for *FGFR3*
    - Targeted NGS to analyze SNP, insertions and deletions, CNVs, gene fusions across 50 cancer-related genes, using DNA and RAN extracted from FFPE samples with target enrichment using multiplex PCR with a panel of gene-specific primers that target key cancer-associated genes

Results

- 7 cases primary lung cancer with clear cell morphology and *FGFR3::TACC3* gene rearrangement
- 4 F, 3 M; 47-81 years (mean 68)
- Peripheral/subpleural localization; 1.4 – 6.5 cm (mean 4.1 cm)
- 5 sqcc (1 with sarcomatoid change and 1 with focal glandular component); 2 adenoca (1 with focal lepidic component, 1 with squamoid architecture)
- Clinical f/u available in 5 patients: all treated with surgical excision, 3 received post op chemotherapy, 2 with concurrent radiotherapy, none received any targeted therapy, 4 known smokers
- F/U data: 1 died in 6 mos with widespread metastatic disease; 1 died in 1 year with brain metastasis; 1 alive and well in 4 years; 1 was tumor free after 27 years but died of COVID 19 ifnection; 1 alive and well after 1 year; 2 were lost to f/u

Take Home Points/Discussion

FGFR3::TACC3 gene rearrangement may be a potential targetable driver alteration in NSCLC

Articles for Notation

NEOPLASTIC

Huang S et al. Incorporation of the lepidic component as an additional pathological T descriptor for non-small cell lung cancer: Data from 3335 cases of lung adenocarcinoma. Lung Cancer 2024;189:107472

Background/Purpose:

- To propose a modified pT stage based on the prsence of lepidic component.

Materials and Methods:

- 3,335 surgical patients with pathological stage I (i.e. T1a, b, cN0 or T2aN0) lung ADC
- Factors affecting survival were investigated with RFS and OS using K-M method and Cox regression analyses
- Subgroup analysis based on lepidic ratio (LR)
- Performance of new modified pT stage system (pTm) evaluated by AUC, C-index, reclassification improvement (NRI), and integrated discrimintation improvement (IDI)

Results:

- Presence of lepidic growth (LP+) present in 1,425 (42.7%) of patients and had significantly better RFS and OS (both $p < 0.001$) than LP- patients
- Similar results were seen in pT1a-pT2a subcategory ($p < 0.050$ for all)
- Multivariable Cox analysis showed LP+ as independent prognostic factor for both RFS and OS
- LR was not an independent factor for both RFS and OS in LP+ patients
- 5 year RFS and OS between T1a (LP-) and T1b (LP+), T1b (LP-) and T1c (LP+), and T1b (LP-) and T2a (LP+) were comparable ($p > 0.050$ for all)
- pTm and the current 8^{th} pT independently predicted RFS and OS, and AUCs c-index, NRI, and IDI analysis all showed pTm hold eter discrimination performance than current pT staging for lung ADC

Take Home Points/Discussion: LP can be an additional down-stage T descriptor for stage I lung ADC. However, they did not clarify the threshold for LP to define LP+. Also, we already have been using the tumor size of pure invasion without including the lepidic component, which is not addressed in the manuscript….

Zhang C et al. Extent of surgical resection for radiologically subsolid T1N0 invasive lung adenocarcinoma: When is a wedge resection acceptable?

Background/Purpose

- The outcome of patient with radiologically peripheral cT1N0 subsolid invasive lung ADC treated with wedge resection is not well known
- This study evaluated when a wedge resection is acceptable in these patients

Materials and Methods

- Retrospective review of patients with peripheral cT1N0 solitary subsolid invasive lung ADC who received sublobar resection
- Clinicopathologic characteristics, 5 year RFS, 5 year lung cancer specific OS were analyzed
- Cox regression model was used to evaluate risk factors for recurrence

Results

- Wedge resection (n=258) and segmentectomy (n=1,245)
- Ground glass nodule (GGN) $\leq$ 2 cm and consolidation-tumor ratio (CTR) > 0.25 and $\leq$ 0.5 had comparable 5-year RFS (96.89%) to that of GGN < 2 cm and CTR $\leq$ 0.25 (100%) (p = .231);
- GGN > 2-3 cm and CTR $\leq$ 0.5 had a significantly lower 5 year RFS (90.12%) compared to 100% in GGN < 2 cm and CTR $\leq$ 0.25 group (p = .046)
- GGN $\leq$ 2 cm and CTR > 0.25 and $\leq$ 0.5 with wedge resection vs. segmentectomy: 5-yr RFS 97.87% and 5-yr OS 92.86% vs. 97.73% and 92.86, respectively; p = .199
- GGN >2-3 cm and CTR $\leq$ 0.5 group with wedge resection was significantly lower in 5-yr RFS than in with segmentectomy (90.61% vs. 100%; p = .043)
- STAS, VPI, and nerve invasion are independent risk factors for recurrence of GGN >2-3 cm and CTR $\leq$ 0.5 by multivariable Cox regression analysis

Take Home Points/Discussion: Wedge resection may be acceptable for those with invasive lung ADC if GGN $\leq$ 2cm and CTR $\leq$ 0.5, but not for > 2-3 cm and CTR > 0.5

Hernandez S et al. Efficient identification of patients with NTRK fusions using a supervised tumor-agnostic approach. Arch Pathol Lab Med 2024;148

Background/Purpose:

- *NTRK* rearrangments as "tumor-agnostic" predictive biomarker for NTRK inhibitors
- Very low frequency of *NTRK* fusions (< 1%) makes it hard to find the eligible patients
- This study is to apply some triaging strategies for a more efficient identification of *NTRK* fusions in a single institution for a practical insight in searching *NTRK* fusions

Materials and Methods

- A strategy combining histologic (secretory ca of breast and sal gland; PTC, infantile fibrosa) and genomic (driver-negative NSCLC, MSI-H colorectal ADC, wild-type GIST) triaging

Results

- IHC with VENTANA pan-TRK EPR17341 assay as a screening method
- All positive IHC cases were simultaneously studied by 2 NGS tests (Oncomine Comrehensive Assau v3 and FoundationOne CDx).
- The detection rate of *NTRK* fusion was 20 times higher (5.57%) by only screening 323 patients thant the largest cohort in the literature (0.30%) based on several 100k patients

Take Home Points/Discussion: Multiparametric strategy (i.e. supervised tumor-agnostic approach) is an effective approach in search for *NTRK* fusions

NON-NEOPLASTIC

Hua JT et al. Characterizing lung particulates using quantitative microscopy in coal miners with severe pneumoconiosis. Arch Pathol Lab Med 2024;148:327-335

Background/Purpose:

- To explore polarized light microscopy coupled with image-processing software termed quantitative microscopy-particulate matter (QM-PM) as a tool to charactrize in situ dust in lung tissue of US coal miners with progressive massive firbrosis, to overcome the limitations of current qualitative assessement or scanning EM with energy-dispersive spectroscopy (SEM/EDS)

Materials and Methods

- Standardized protocol using microrscopy images to charactrize the in situ burden of birefringent crystalline silica/silicate particles (mineral density) and carbonaceous particles (pigment fraction)
- Mineral density and pigment fraction were compared with pathologists' qualittive assessements and SEM/EDS analyses
- Particle features were compared between historical (born before 1930) and contemporary coal miner, who likely had different exposures given the different mining technology

Results

- 85 coal miners (62 historical, 23 contemporary) and 10 healthy controls
- Mineral density and pigment fraction by QM-PM technology were comparable to consensus pathologists' scoring and SEM/EDS analyses
- Contemporary miners had greater mineral density than historical miners (186 456 vs. 63 727/ mm^3; p = .02) and controls (4542/mm^3), c/w higher silica/silicate dust.
- Contemporary and historical miners had similar particle sizes (median area, 1.00 vs 1.14 μm^2; p = .46) and birefringence under polarized light (median grayscale brightness 80.0 vs 87.6; p = .29)

Take Home Points/Discussion: QM-PM reliably characterized in situ silica/silicate and carbonaceous particles in a reproducible, automated, accessible, and time/cost/labor-effiient manner!

REVIEWS

Mukhopadhyay S. Differential diagnosis of IgG4-positive plasma cells in the lung. Seminar Diag Pathol 2024;41:72-78: Entire issue is dedicated to IgG4-related disease. Worth checking out!

Bateman AC et al. Challenges and pitfalls in the diagnosis of IgG4-related disease. Seminar Diag Pathol 2024;41:45-53

Hofman P et al. Current challenges and practical aspects of molecular pathology for non-small cell lung cancers. Virchows Archiv 2024;484:233-246: Addresses current predictive biomarkers and algorithms for use in thoracic oncology molecular pathology as well as the central role of the pathologists, notably in molecular tumor board and their particpation in the tx decision making

O'Leary CL et al. Immune related toxicity in NSCLC: current state-of-the-art and emerging clinical challenges. J Thor Oncol 2024;19:395-408

EDITORIAL/CASE REPORTS

Frank AL et al. A multidisciplinary review of several aspects of asbestos-related lung cancer (ARLC). Lung Cancer 2024;189:107474

Ufuk F et al. Case 323: Minute pulmonary meningothelial-lke nodules. Radiology 2024;310:e222512

Wang H et al. Late-onset diffuse lung disease in an 8-year-old girl. Chest 2024;165:271-e74

George GV et al. ALK-rearranged CD30-positive poorly differentiated lung adenocarcinoma, mimicking anaplastic large-cell lymphoma. Histopathology 2024;84:900-902