Pulmonary Journal Club May 2022, (Articles from April 2022)

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Articles for Discussion

Kishikawa S, et al., Distinct properties of pure- and mixed-type high-grade fetal lung adenocarcinomas by genetic profiling and transcription factor expression. Virchows Archiv (2022) 480:609–619. https://doi.org/10.1007/s00428-021-03247-7.

Background: Rare - 0.3–1.4% of all lung adenocarcinomas

Complex, branch forming tubular glands lined by non-ciliated columnar or cuboidal cells. simulating fetal lung tubules in the pseudoglandular stage (5–17 weeks of gestation); glands densely packed and situated within loose to moderate cellular fibroblastic stroma. Clear cytoplasm (supranuclear and/or subnuclear vacuoles ~early secretory endometrium) rich in glycogen.

LG FLAC: Homogenous (100% pure), low nuclear atypia and prominent morule formation Morules - cell clusters composed of spindle- to oval-shaped cells, and they lack cellular atypia or mitotic figures. Recurrent *CTNNB1* mutations leading to aberrant nuclear accumulation of β-catenin. Usually young to middle-aged women

HG FLAC: At least 50% fetal morphology, prominent nuclear atypia, often nucleoli, no morule formation. Mitoses and necrosis common. Associated with other conventional types of lung adenocarcinoma (lepidic, papillary, acinar, micropapillary, and solid patterns). B-catenin membrane-localized. Predominantly occurs in elderly men

Unanswered questions:

- Prevalence of high-grade fetal adenocarcinomas
- Is high-grade fetal adenocarcinoma a distinct variant of lung adenocarcinoma
- Clinicopathological differences among pure-type high-grade fetal lung adenocarcinoma and those with fetal lung-like components admixed with conventional adenocarcinoma cells (mixed-type high-grade fetal lung adenocarcinoma) remain undetermined.

Most of the global literature regarding fetal lung adenocarcinoma comprises case reports and case series This case series had fewer numbers than some others

Objectives: to determine the prevalence and the genetic and transcriptional characteristics of LG, pure- and mixed-type HG FLAC.

Methods: Screened the archives of the Department of Human Pathology, Juntendo University School of Medicine, Tokyo, Japan, for all patients who had undergone complete resection of primary lung carcinoma between January 2010 and December 2020. 3895 cases, two pathologists (SK and TH) reviewed the slides, and made pathological diagnoses based on the

2021 WHO classification. Performed IHC, molecular analysis (NGS and nanostring for kinase)
Didn't macrodissect out the mixed tumors

Results

Clinicopathological findings of lung adenocarcinoma with fetal lung-like morphology

11 (0.3%) had components of fetal lung-like morphology

- "Pure-type high-grade fetal adenocarcinoma were younger than those with mixed-type high-grade fetal adenocarcinoma. The other clinicopathological findings were similar". N of 2, with one 41 y and one 74 y old.
- "Pure-type high-grade fetal adenocarcinoma exhibited considerably higher mitotic counts than the other categories, including low-grade fetal lung adenocarcinoma". N of 2
- Tumor necrosis was observed in most cases – LG FLC only comedo-like necrosis and no geographic necrosis

IHC: excluding that for β-catenin, SMARCA4, and SMARCB1, considered samples positive when **> 1%** of the tumor cells were stained. For β-catenin – a nuclear pattern in ≥ 10% Oncofetal markers: glypican-3 one case of pure-type HG FLAC and in two cases of mixed-type HG FLAC. Only one case of mixed-type HG FLAC was positive for AFP. Loss of SMARCA4 and SMARCB1 expression was not detected in all cases

Molecular analyses

- no mitogenic driver mutations in pure-type HG FLAC
- *CTNNB1* mutations in both LG FLAC and one mixed-type HG FLAC and all had nuclear β-catenin accumulation ((in fetal and non-fetal components) as well as membranous staining
- All cases were positive for membranous E-Cadherin
- No kinase fusions detected
- No MSI

Conclusions:

- the prevalence of pure-type high-grade fetal lung adenocarcinoma may be as high as that of low-grade fetal lung adenocarcinoma. Based on an n of 2
- No genetic alterations including *TP53*, *CTNNB1*, and *APC* mutations that could activate Wnt signaling were identified in the pure-type HG FLAC.
- Mixed-type HG FLAC and lung adenocarcinoma with HG fetal features frequently express TTF-1.
- Pure-type HG FLAC lacked TTF-1 expression but expressed CDX2 - similar to a previous study: (4 cases of pure HG FLAC; Suzuki et al. High-grade fetal adenocarcinoma of the lung is a tumour with a fetal phenotype that shows diverse differentiation, including high-grade neuroendocrine carcinoma: a clinicopathological, immunohistochemical and mutational study of 20 cases. Histopathology 2015, 67:806–816)

- Mixed-type HG FLAC as well as lung adeno with HG fetal features may fundamentally constitute conventional lung adenocarcinoma with a characteristic morphological pattern rather than a specific tumor entity.
- Pure-type HG FLAC is genetically and transcriptionally distinct from mixed-type HG FLAC

Potential limitations:

- small number of patients limited observation time for survival, and the
- small numbers of genes analyzed using NGS, although did analyse most driver mutations that have been found to be positive in HG FLAC in previous papers (Suzuki et al. Comprehensive molecular analysis of genomic profiles and PD-L1 expression in lung adenocarcinoma with a high-grade fetal adenocarcinoma component. Transl Lung Cancer Res. 2021 Mar;10(3):1292-1304).

Yang SR, et al., Diagnosis of Hypersensitivity Pneumonitis. Review and Summary of American College of Chest Physicians Statements. Am J Surg Pathol 2022;46:e71–e9.

Assessment of lung biopsies for the diagnosis of HP is one of the most challenging diagnostic problems for surgical pathologists.

- May be difficult or impossible to pinpoint the offending antigen, even with an extremely detailed history,
- Also reflects a lack of consensus about the clinical, radiologic, and pathologic features of HP.

This review explores the multidisciplinary diagnostic evaluation of HP with a focus on the pathologic features as outlined in the most recent CHEST guidelines. (Fernández Pérez ER, Travis WD, Lynch DA, et al. Diagnosis and evaluation of hypersensitivity pneumonitis: CHEST Guideline and Expert Panel Report. Chest. 2021;160:e97–e156)

PATHOLOGIC FEATURES

- HP can present with a wide range of histologic patterns depending on the clinical course and disease progression
- "Nonfibrotic HP" (cellular HP) and "Fibrotic HP" patterns
- While peribronchiolar metaplasia is a nonspecific reaction to bronchiolar and peribronchiolar injury, widespread involvement affecting >50% of bronchioles should raise the consideration for fibrotic HP

In the CHEST guideline, the histologic criteria are divided into 4 diagnostic categories:

(1) *Typical* nonfibrotic HP or fibrotic HP

(2) *Compatible with* nonfibrotic HP or fibrotic HP

(3) *Indeterminate* for nonfibrotic or fibrotic HP

(4) *Alternative Diagnosis*

CHALLENGES IN MULTIDISCIPLINARY DIAGNOSIS OF HP

"some patients with typical features of fibrotic HP on CT may show only NSIP or UIP pattern on biopsy – is diagnosis fibrotic HP? (assuming no antigen identifiable); conversely, some patients with typical NSIP or UIP pattern on CT may have histologic findings of HP" is diagnosis fibrotic HP?

COMPARISON WITH ATS/JRS/ALAT HP GUIDELINE: Am J Respir Crit Care Med. Raghu et al. 2020 Aug 1;202(3):e36-e69.

- Instead of "compatible" ATS uses "probable HP" - the constellation of histologic findings in this category are nonspecific and outside of a clinical and radiologic picture favoring HP, this pattern by itself does not indicate that the diagnosis is probably HP
- ATS "indefinite" classification is based on inclusionary criteria and requires the presence of select histologic features that are characteristic for HP (eg, bronchiolocentric cellular interstitial pneumonia or cellular bronchiolitis)
- CHEST indefinite criteria do not require specific features other than an ILD pattern that does not meet the criteria for typical /compatible with HP, or an alternative diagnosis - broader and more open-ended indeterminate category, which could be more clinically applicable given that this inconclusive category essentially represents a "wastebasket" category rather than a specific disease entity with well-defined histologic features
- ATS criteria include bridging fibrosis (pattern of fibrosis that spans bronchioles to the pleura or interlobular septa) as a diagnostic feature under airway-centered fibrosis for fibrotic HP but bridging fibrosis can be seen in UIP/IPF
- CHEST guideline separates the diagnostic criteria into major and minor features to emphasize the critical histologic findings supporting HP
- CHEST guideline highlights the importance of the chronic inflammation that is seen around alveolar ducts and not just bronchioles (cellular bronchiolitis) for the diagnosis of HP.
- Although both guidelines include UIP-pattern as an example of diffuse fibrosing interstitial pneumonia that can be seen in fibrotic HP, the ATS guideline doesn't refer to the "UIP" terminology

SLB

CHEST guidelines and expert panel report **suggest considering histologic lung biopsy "when all available data … do not yield a confident diagnosis and results may help guide management"** but qualified as "weak" recommendations with "very low-quality evidence."

- While "typical" features of nonfibrotic HP may occasionally be present in cases of fibrotic HP, particularly if >1 site is sampled, 25% to 30% of cases will lack identifiable granulomas or giant cells. Does this mean the majority, >70% will have granulomas or giant cells?

- Peribronchiolar metaplasia, particularly when present in over 50% of airways or greater, is a helpful diagnostic clue generally favoring HP when present.
- BUT
- In one study, 33% of patients with clinical IPF were found to have significant bronchiolocentric fibrosis.
- Fibrotic HP with a pure UIP-pattern tends to have fewer fibroblast foci than IPF.

BAL

- BAL fluid lymphocyte level appears to vary inversely according to fibrosis burden in the lung.
- the lack of BAL lymphocytosis may help exclude nonfibrotic HP in the appropriate clinical context, it **does not rule out fibrotic HP**.
- Marked BAL lymphocytosis with **40% to 60% lymphocytes**, although not specific, is highly suggestive of HP in the appropriate clinical and radiology context and can help exclude competing causes such as IPF

CTD-ILD and HP may be histologically indistinguishable – plasma cells the main clue?

ILD with features of HP can also occur in up to 10% of patients with **pleuroparenchymal fibroelastosis (PPFE).** Biopsies show features of nonfibrotic HP or fibrotic HP in addition to PPFE, and CT also shows characteristic findings of PPFE and HP

Rosen Y, et al., Pulse/hyaline ring granuloma revisited: etiology role of seed-derived storage cells. Virchows Archiv 2022 March; 480: 499-508. https://doi.org/10.1007/s00428-021-03222-2.

Fumio I, et al., Seed storage cell granuloma: a nostalgic look back on pseudotuberculosis. Virchows Archiv 2022 January; 480: 715-716. https://doi.org/10.1007/s00428-021-03258-4

Botany:

- The human diet contains a variety of seeds, e.g., peas, beans, corn, rice, lentils, and nuts.
- Seeds are produced by
  gymnosperms (conifers and others)
  angiosperms (flowering plants) – most of the seeds humans consume
- Two major components of seeds are the embryo and storage cells that provide nutrition to the embryo.
- Storage cells contain the nutrients starch, protein, and lipid in varying amounts with starch usually predominating
- A **legume** is a plant in the family Fabaceae (or Leguminosae), or the fruit or seed of such a plant.

- Seeds of legumes are encased within a pod and include beans, peas, chickpeas, lentils, soybeans, carob, and peanuts.
- **Lentils** are legumes, the edible seeds of plants of the genus **Lens** of the legume family.
- **Non-legumes** with storage cells, such as corn, tomato, rice, and almonds can also incite granulomatous responses and may not be able to distinguish from those due to legumes
- **Pulse,** derived from the Latin words "puls" or "pultis" meaning "thick soup"a subgroup of the legume family
- Pulse, as defined by the United Nations Food and Agriculture Organization (FAO), is reserved for legume crops harvested solely for the **dry seed** - commercial agricultural term

History:

- Society of Physicians in Zurich in 1890, demonstrated a case of perforated gastric ulcer with miliary tubercles of the peritoneum, "**Pseudotuberculosis**" containing pear seeds. (*Hanau, (1891) Einen Fall von Ulcus ventriculi rotundum mit geheilter localisirter Perforationsperitonitis. Corr-Bl Schweiz Arzt 21:207–209*)
- The first pathology case series of **lung** was published in **1942** (*Fetterman GH, Moran TJ (1942) Food aspiration pneumonia PA Med J 45:810–812*)
- "**Giant-cell hyaline angiopathy**" (*Dunlap CL, Barker BF (1977) Giant-cell hyalin angiopathy. Oral Surg Oral Med Oral Pathol 44:587–591*). 7 oral lesions in edentulous patients thought to be a vasculitis – subsequent EM and IHC showed these structures are not vessels
- "**Pulse granuloma**" (*King OH (1978)* "Giant cell hyaline angiopathy": pulse granuloma by another name?
- "**Hyaline ring granuloma**" (*Chou L, Ficarra G, Hansen LS (1990) Hyaline ring granuloma: a distinct oral entity. Oral Surg Oral Med Oral Pathol 70:318–324.*)

Morphology of the granuloma-inciting cells

- large, ovoid, and round cells measuring up to approximately 250 mμ in diameter.
- Outer cell wall composed of cellulose – often birefringent, when well preserved may be PAS positive
- Granules of varying size within their cytoplasm "starch granules" (PAS positive) but they may also contain protein and lipids in varying amounts.
- Inflammatory reaction -multinucleate giant cells that are accompanied by a variety of chronic inflammatory cells as well as varying numbers of polymorphonuclear leukocytes

Aims: To test hypothesis that the PG/HRG-inciting cells are seed-derived storage cells

Methods:

- Prepared microscopic sections from a variety of hydrated and formalin-fixed leguminous and non-leguminous seeds
- Frozen peas, fresh corn, kernels, and tomato were not hydrated prior to processing.

- Sections of chickpeas and white beans were examined microscopically before and after digestion with saliva and alpha amylase (at room temp for 2 weeks)
- Sections stained with H&E, PAS, and iodine.
- Morphology of the PG/HRG-inciting cells depicted in numerous publications compared
- Granules with very high starch content, e.g., those in corn kernels: eosin negative, PAS and iodine positive.
- Post digestion - loss of PAS and iodine staining
- Birefringence of the cellulose cell walls- strong prior to digestion- negative post-digestion

Differential diagnosis

- plant tissue other than seed contents: rectangular or square shapes - typically have a latticework appearance
- amyloid
- healed vasculitis
- parasite

Conclusions: Based upon comparison of the morphology of the granuloma-inciting cells shown in images of PG/HRG in published cases and the authors cases, the granuloma-inciting cells are seed derived storage cells.

Clues to their identity are as follows:

- They maintain the approximate size and shape of the well-preserved cells
- A cellulose cell wall is often visible
- The cells are filled with finely granular or amorphous material unless they are extensively infiltrated with inflammatory cells

Should be termed "**seed storage cell granuloma**"

<u>Articles for Notation -Neoplastic</u>

Reuling EMB, et al., Diagnosis of atypical carcinoid can be made on biopsies > 4mm² and is accurate. Virchows Archiv (2022) 480: 587-593. <u>https://doi.org/10.1007/s00428-022-03279-7</u>.

In the 2021 WHO thoracic tumors, gradation of lung carcinoids in biopsies is discouraged. Authors hypothesized that atypical carcinoid (AC) could be reliably diagnosed in larger preoperative biopsies.

Methods: Biopsy-resection paired specimens of carcinoid patients were included, and definitive diagnosis was based on the resection specimen according to the WHO 2021 classification. A total of 64 biopsy-resection pairs (26 typical carcinoid (TC) (41%) and 38 AC (59%)) were analyzed. In biopsies measuring<4mm2, 15/15 AC (100%) were misclassified compared to 14/23 AC (61%) of biopsies≥4mm2.

Results:
1. If AC was diagnosed in the biopsy, the diagnosis was consistently accurate (9/9, 100%).
- In biopsies measuring < 4 mm2, 15/15 AC (100%) were misclassified as TC
- In biopsies ≥ 4 mm2, 14/23 (61%) AC were misclassified as TC
- Ki-67 in the biopsy was not of additional value to discriminate between TC and AC, irrespective of the biopsy size.

Take home message: results not surprising and reinforces the guidelines of not diagnosing TC on biopsy. If necrosis or increased mitoses are seen on biopsy, is reasonable to suggest may be dealing with AC
Text was difficult to understand at times and tables, figures quite misleading.

Volterrani L, et al., HRCT predictors of GGO surgical resection: Histopathological and molecular correlation in the era of lung sparing surgery. Lung Cancer 2022 Feb; 166: 70-75. https://doi.org/10.1016/j.lungcan.2022.02.001.

Background: Ground-glass pulmonary opacities (GGOs) are increasingly encountered in routine clinical practice and an accurate differentiation between benign and malignant lesions is crucial.. These patients have a favorable prognosis with tendency to develop a second primary lung cancer and a redo-surgery. For this reason, a conservative lung sparing resection should be preferred in order to preserve lung capacity and to allow a second radical resection. **Aim**: to evaluate the relationship between radiological features and the biological behavior of these nodules. Secondary endpoint - to identify any radiological predictors able to choose the type of surgical resection and the extent of lymphadenectomy.

Materials and Methods: This single-center retrospective study included all patients, who underwent HRCT) and surgical resection for GGOs 2010 - 2020.

Results: 56 patients enrolled, 65 lesions (15 pure GG and 50 part-solid) resected. A direct significant correlation was found between: the GG at HRCT and the amount of lepidic component ($p < 0.0001$; $R = 0.305$), the tumor grading and the lepidic component at HRCT ($p = 0.003$), the percentage of GG and the expression of Ki67 ($p = 0.016$), the lepidic percentage and the expression of Ki67 ($p = 0.004$; $R = 0.223$).
Difference between GGOs diameter on preoperative HRCT and after histopathological analysis: a mean reduction of GGO diameters at histopathological evaluation of about 35% compared to the same diameters at HRCT. This is due to a higher presence of air in the alveoli which stretches the parenchyma, altering the GGO size.

Conclusion: Pure and part-solid GGOs could benefit from less invasive and lung sparing surgery with just nodal sampling. The major limitations are the number of patients and the lack of a longer follow-up.

Take home message: Not much new here. The correlation R values are relatively low. The significant correlation between GG percentage and proliferation index is not explained.

Ilié M, et al., Analytical validation of automated multiplex chromogenic immunohistochemistry for diagnostic and predictive purpose in non-small cell lung cancer. Lung Cancer 2022 April; 166: 1-8. https://doi.org/10.1016/j.lungcan.2022.01.022

Background: Increasing numbers of diagnostic and predictive markers required for precision thoracic oncology but tissue is limited. Multiplex immunohistochemistry (mIHC) maximizes tumor tissue preservation. Authors optimized two automated four-plex assays on a commercially available IHC autostainer i) TTF1, p40, PD-L1, CD8; and, ii) ALK, ROS1, BRAFV600E, NTRK. **Aims**: to evaluate the repeatability and concordance of the assessment of the cell density.

Methods: Intra-site repeatability was evaluated on serial tumor sections from non-small cell lung carcinomas (NSCLC). The concordance was assessed by linear fit to plots of the percentage staining evaluated on tumor sections from 89 NSCLC patients. Interpretation was assessed blindly by three trained thoracic pathologists (MI, VH, and PH).

Results: Average concordance for a staining rate of 95.4% was achieved between conventional IHC and mIHC across all selected markers.

Conclusions: Optimized mIHC assay gave a sensitive and repeatable assessment of two panels of eight diagnostic and predictive biomarkers for NSCLC.

Take Home Message: I see some value in multiplexing but only some cases will need all 4 of the first panel and NGS can obviate the need for the second panel.

<u>Case Reports</u>

Clark SM, et al., A 31-Year-Old Man with Asthma, Chest Discomfort, and Shortness of Breath. Chest 2022; 161(4): e209-e212. https://doi.org/10.1016/j.chest.2021.10.035.

Takahiro S, et al., Bronchial Cast Hiding Pulmonary Tuberculosis. Am J Respir Crit Care Med 2022 April: 205(8): e16-e17.

Chieng H, et al., A 24-year-old man with Dyspnea and a broken left femur. Chest 2022 April; 161(4): e225-e231. https://doi.org/10.1016/j.chest.2021.10.026.

Tripathi A, et al., A 58-year-old man with Hemoptysis after COVID-19 infection. Chest 2022 April; 161(4): e213-e217. https://doi.org/10.1016/j.chest.2021.11.024.

Tomiyasu S, et al., A 70-Year-Old Woman With Long-Term Nonresolving Pneumonia. Chest 2022 April; 161(4): e219-e223. https://doi.org/10.1016/j.chest.2021.10.019.

Steenhuis EGM, et al., A 20-Year-Old Woman With Thoracic Pain and Progressive Dyspnea on Exertion. 2022 April; 161(4): e233-e240. https://doi.org/10.1016/j.chest.2021.11.011.

Wong GS, et al., A 73-Year-Old Woman With Progressive Shortness of Breath and Right-Sided Hemithorax Opacity. Chest 2022 April; 161(4): e203-207. https://doi.org/10.1016/j.chest.2021.10.039.

Reviews

de Waal AM, et al., Lung epithelial cells interact with immune cells and bacteria to shape the microenvironment in tuberculosis. Thorax 2022; 77: 408-416. doi:10.1136/thoraxjnl-2021-217997.

The lung epithelium has long been overlooked as a key player in tuberculosis disease. In addition to acting as a direct barrier to Mycobacterium tuberculosis (Mtb), epithelial cells (EC) of the airways and alveoli act as first responders during Mtb infections; they directly sense and respond to Mtb by producing mediators such as cytokines, chemokines and antimicrobials. Interactions of EC with innate and adaptive immune cells further shape the immune response against Mtb. These three essential components, epithelium, immune cells and Mtb, are rarely studied in conjunction, owing in part to difficulties in co-culturing them. Recent advances in cell culture technologies offer the opportunity to model the lung microenvironment more closely. Herein, we discuss the interplay between lung EC, immune cells and Mtb and argue that modelling these interactions is of key importance to unravel early events during Mtb infection.