Pulmonary Journal Club September 2023 (Articles from August 2023)
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<u>**Articles for Discussion**</u>

1. **Lami K., et al., Overcoming the Interobserver Variability in Lung Adenocarcinoma Subtyping: A Clustering Approach to Establish a Ground Truth for Downstream Applications. Arch Pathol Lab Med 2023, 147, 885-895. doi: 10.5858/arpa.2022-0051-OA**

Background:

- The accurate identification of different lung adenocarcinoma (ADC) histologic subtypes is important as they are independent prognostic factors for relapse-free and overall survival.
- There is considerable interobserver variability even amongst experts. *Cluster analysis* is a method of identifying relevant subgroups of items through statistical analysis to divide a group of entities into more uniform and mutually exclusive groups based on their correlations.
- Lung ADC subtyping, with its known interobserver variability, can benefit from cluster analysis by refining the diagnostic criteria and obtaining the ground truth.
- Several deep learning algorithms to recognize certain subtypes of lung ADC have been developed with the training set based on annotated ground truths obtained from 1 to 3 pathologists.
- A set of consensus images of lung ADC subtypes was created to try to decrease the interobserver variability.
- These images could then be further used as the ground truth to train CNNs for automatic detection and classification of lung ADC subtypes, improving their diagnostic accuracy.

Aim: To provide an overview of the diagnostic agreement for ADC subtypes among pathologists and to create a *ground truth* using the clustering approach for downstream computational applications.

Methods: Used a series of cases from a single institute (2007 – 2020). Three sets of lung ADC images with different evaluation levels (small patches, areas with relatively uniform histology, and whole slide images) were reviewed by 17 expert lung pathologists and 1 pathologist in training.

Figure 1 - overall workflow.

- Electronic medical records: retrieved 191 representative surgically resected lung ADC cases encompassing all histologic subtypes.
- Each glass slide was scanned and produced 330 WSIs.
- Cases were divided into 3 sets which each had a different evaluation method.
- First set: the dominant pattern of 4702 small patches was recorded. Patches of the first set were reviewed using an application for smartphones/tablets created explicitly for this purpose - *are cellphone screens the best way to review digital images?*
- Second set: the dominant subtype of annotated areas representing relatively uniform lung ADC histology was recorded. Subtypes included lepidic, acinar, papillary, micropapillary, solid, invasive mucinous ADC, other cancer types (complex glandular pattern, cribriform pattern,

colloid ADC, fetal ADC, or enteric ADC), and no carcinoma cells (for patches of the first set not visibly containing any cancer cells). Invasion was defined as a lung ADC subtype other than the lepidic growth. The 79 WSIs of the second set were uploaded to the PathPresenter platform and a spreadsheet was created to help pathologists sort out each annotated area with the estimated lung ADC subtypes

- Third set: every subtype present in the entire slide of each of the 239 WSIs was recorded. 15 pathologists provided a case-level diagnosis of the 142 patients by determining the dominant and minor subtypes of lung ADC for each patient and their estimated percentages in 5% increments.
- Older glass slides were restained *Important point*
- Stats:
  - Cohen κ coefficient - evaluation of the pairwise agreement for invasive cancer versus noninvasive cancer patches and cancer versus noncancer patches, with a total of 153 κ values calculated for each category from different combinations of the 18 pathologists.
  - Fleiss κ coefficient: evaluation of the overall and histologic subtype agreements from multiple raters. Agreements were defined as poor, slight, fair, moderate, substantial, and almost perfect for κ values of less than 0, 0.01 to 0.20, 0.21 to 0.40, 0.41 to 0.60, 0.61 to 0.80, and 0.81 to 1.0, respectively.
  - Ward method - hierarchical cluster analysis with R software from labels of the 4702 patches of the 18 pathologists; distance between pathologists was determined by the Cramér V.
  - Uncertainty in the result from the clustering analysis was assessed via multiscale bootstrap resampling.

Results:

- First set, (**Figure 2**) 37% had an overall consensus among all pathologists, including 1520 patches labeled as "no carcinoma cells" . The overall Fleiss κ score for the agreement of all subtypes was 0.58 *("moderate").*
- Solid pattern - most consensus patches, all 18 pathologists having complete agreement on 180 patches, followed by invasive mucinous (29 patches), acinar (8 patches), and micropapillary (5 patches) subtypes.
- No complete consensus on lepidic, papillary, and other carcinoma types
- Pathologists were hierarchically grouped into 2 clusters, with κ scores of 0.588 and 0.563 in clusters 1 and 2, respectively. Figure 4
- After *excluding outlier pathologists*, - retained 12 pathologists for the evaluation
- In the third set, survival analysis was conducted to evaluate the ability of pathologists and clusters to separate noninvasive-predominant from invasive-predominant tumors – *but the morphology of the tumor is not the only determinant of survival*
- Patches from the first 2 sets that obtained the consensus of the 18 pathologists - "consensus patches" - *ground truth* of lung adenocarcinoma subtypes.
- Pairwise agreements for invasive versus noninvasive patches - 0.05 to 0.76
- Agreements for cancer versus noncancer patches had the highest agreement among pathologists

Conclusion:

- The marked variation in interobserver agreement for invasive versus noninvasive patterns is worrying as the size of the invasive component is a determinant of the T stage of lung ADC.

- Reasons for the relatively low agreement for differentiating cancer from noncancerous patches explained by difficulty in distinguishing macrophages or reactive epithelial cells from cancer, and fatigue due to having to sort a considerable number of patches.
- The solid pattern had the most consensus patches similar to other studies
- Acinar and papillary patterns had the lowest agreements in the first set.
- Allowing pathologists the ability to magnify each slide up to 20x mag and to view whole slides didn't improve agreement
- Agreements of the third set were slightly lower than the second set. The "other carcinoma" subtype showed the worst agreement among the lung ADC subtypes in the third set and thought to be due to misclassification of cribriform or complex glandular patterns as other carcio. This is was explained as being due to the misrecognition of cribriform or complex glandular patterns; There was also possible intraobserver variability.
- The agreements obtained with close scrutiny of small patches (first set) were superior to the ones obtained with analyses of an enclosed area in a WSI (second set) or the entire WSI (third set) ?inspection of small areas with minimal morphologic features at low power results in better agreements when evaluating lung ADC subtypes.
- The selection of the clustering approach over the majority rule is explained by the fact that the cluster analysis creates subgroups of pathologists with distinctive diagnostic criteria for lung ADC subtypes, which can help to refine ground truth images.
- Also, an 80% agreement rule was abandoned as it resulted in few consensus patches.
- The pathologists' answers from the first set created 2 hierarchical clusters, with the main difference seen principally in 1 of the 12 cases included for evaluating the pathologists' agreement. Cluster 1 agreed with an invasive mucinous ADC–predominant cancer, whereas cluster 2 favored micropapillary and other cancer subtypes. *What about a mucin stain to determine the 'ground truth'?*
- After validation with survival analysis, the whole set of ground truth images will be made publicly accessible.

Limitations:

- Only 49 cases from a single institute were used to obtain consensus patches. More infrequent ADCs, e.g. colloid or fetal, were not sufficiently represented.
- The annotation of the second set was done by a trainee pathologist. However, the agreement of the third set revealed a similar score to that obtained from the second set. This shows that the agreement was still low, even if pathologists had the freedom to evaluate the WSIs without limitations.
- The consensus patches retrieved from the 2 clusters were not demonstrated to improve the overall agreement. Although the clustering approach resulted in better identification of invasion than some pathologists alone, consensus patches themselves were not used to evaluate the improvement of lung ADC subtype recognition. This issue needs to be addressed in a separate study, in which consensus patches can be used to train CNN models.

Take Home Message: Interobserver agreement surprisingly poor and this limits the ability of this method to provide 'ground truth' images for future training sets and this paper makes me doubt the validity of these images. Heavy reliance on trainee pathologist not ideal for something that is recognised to be a difficult area. This area is still very new and fraught with difficulties.

2. Ding H, et al. Deep learning-based classification and spatial prognosis risk score on whole-slide images of lung adenocarcinoma. Histopathology 2023; 83: 211-228. https://doi.org/10.1111/his.14918

Introduction

- Understanding the heterogeneity of lung adenocarcinoma is critical for precision medicine to tailor cancer treatments for patients.
- accurate histological diagnosis of histological subtypes is labour-intensive and time-consuming, requiring experienced pathologists
- **Hypothesis**: that a DL model will aid pathologists in identifying and quantifying the proportions of histological subtypes and extracting the spatial features of each one which will, in turn, enhance the prognosis of LUAD patients

Methods

Figure 1: Overview of the workflow

- 1. established well-annotated WSI data sets (ACHNJMU-1) containing 40 000 path-level tiles derived from 460 single-lesion WSIs from 129 patients. 5 main subtypes: lepidic (LPA), acinar (APA), papillary (PPA), micropapillary (MPA) and solid (SPA) adenocarcinoma *Was whole tumor sampled, what percentage?*
- 2. LUAD subtype classification models based on classic DL architectures were built and trained (LUAD-subtype deep learning model (LSDLM)).
- 3. The LSDLM was evaluated on internal and two independent external WSI testing data sets (ACHNJMU-2, THHTCM, TCGA).
- 4. A transfer test was performed on the Cancer Genome Atlas (TCGA) data set
- Pathologists with 3 and 8 years of work experience reviewed the H&E slides and annotate the digitalised WSI data sets. When the evaluations conflicted, another pathologist with 20 years of work experience made the call
- Tile-level prediction performance of the LSDLM on a histologically mixed WSI data set (ACHNJMU-3) was close to the level of the senior pathologist.
- The WSIs from TCGA were also used to evaluate models and excluded were those whose predominant subtype was too ambiguous to determine or not in the five subtypes mentioned (*excluded the most difficult cases*)
- To evaluate the prognostic ability of the DL model, LUAD RNAseq data sets from 13 independent studies were downloaded from the Gene Expression Omnibus (GEO; ncbi.nlm.nih.gov)

Results

- Using the DL model, F1-scores of 0.89 (ACHNJMU-2), 0.91 (THTCM) and 0.71 (TCGA)
- Figure 2A, *3rd image at top ?not acinar but cribriform?*
- Four lesions (two APAs and two LPAs) were mistaken by the AI as MPAs, and two (one LPA and one APA) were misidentified as SPAs
- LSDLM tended to mistakenly identify lower-risk LUAD subtypes (LPA, APA) as higher-risk subtypes (MPA, SPA). Considering the poor prognosis of MPA and SPA, a few false positives were deemed to be acceptable in clinical diagnosis procedure
- One slide was misrecognised as SPA, which resulted from an atypical pattern of PPA that confused the model

- LSDLM accuracy relatively low on the TGCA data set- Reasons for poor performance: variations in staining colour and slide quality, a complex APA pattern was found among the 18 WSIs, which can be difficult to distinguish from SPA
- A survival-related risk score was calculated, RS based on tile-level testing results from the TCGA data set, which contains available survival information
- The geospatially related K-RS was established by combining the prediction of the LSDLM with the K score generated from the geospatial location of tiles in the whole tumour region.
- The geospatial location of the individual pathological subtypes affected the prognosis modestly(Figure 6) although the worse patterns, SPA and MPA weren't affected.
- Gene-level differential analysis was conducted between 21 high and 75 low risk patients from the TCGA data set; 299 up-regulated genes in the high group were selected as signatures associated with histological features of high-risk pathological subtypes (AI-SRS), and an AI-SRS score (AI-SRSS) was constructed.
- The performance of AI-SRSS was evaluated, - the SPA has the highest AI-SRSS in the three data sets, LPA has the lowest AI-SRSS and PPA, APA and MPA were in the middle "in accordance with the risk level of five different subtypes". *MPA in fact is regarded as having a higher risk*

Conclusions: In all three datasets, the LSDLM showed a high-level capacity to distinguish LUAD subtypes. The geospatially related risk score, K-RS showed moderate improvement in patient prognosis stratification compared with the RS score result derived solely from pathological subtypes. Additionally, the spatial histopathological gene expression signature of AI-SRSS according to the K-RS score was established and verified as an independent prognosis factor in the public data sets.

Has previously been shown that cancer subclones derived from immune "cold" regions were more closely related in mutation space, diversifying more recently than subclones from immune hot regions, therefore the K-RS score may have potential value and needs further investigation.A potential relationship between the gene-level signature and the pathological characteristics of the tissue, was found. DL models based on histopathological images can be considered as a complementary approach for analysing potential features that cannot be adequately discovered by bulk sequencing.

Limitations:

- Limited scale of the data sets. 460 WSIs from ACHNJMU, 23 WSIs from the THTCM and 96 WSIs from TCGA;the scale of the data sets is not adequate to train a DL model which is stable enough to apply in real-world circumstances. More WSIs should be included to form a larger, well-annotated data set.
- Several newly reported high-grade patterns, such as the cribriform and other complex glandular patterns that were previously classified as APA, were not included
- No examination of the micro-environment and tumor interaction.

Take Home Message: DL is here to stay and hopefully will become a useful tool for pathologists. The 'black box' nature of the methodology makes it difficult to assess how useful each model is – the use of survival data has many confounding factors. The accuracy of the initial annotation is of great importance given that the pathologist is the (present) 'gold standard', therefore the use of inexperienced pathologists to do the tedious work, is not ideal

3. Zheng Q, et al., Frequent EGFR exon 20 insertion in the so-called peripheral-type squamous cell neoplasm of uncertain malignant potential: a variant of bronchiolar adenoma or under-recognised entity? Histopathology 2023, 83, 178-192. DOI: 10.1111/his.14890.

Background

- There is increased resection of benign lung nodules due to the adoption of low-dose CT screening and VATS: bronchiolar adenoma (BA)/ ciliated muconodular papillary tumour (CMPT), pulmonary papillomas, squamous (SCP), glandular (GP), and mixed squamous cell and glandular papilloma (MP)
- The authors came across a rare intrapulmonary peripheral type squamous cell tumour, "peripheral type squamous cell neoplasm of uncertain malignant potential (PSCN-UMP)"
- BAs all have a continuous layer of basal cells (p40 and CK5/6-positive) and can be subtyped, based on morphologic and immunohistochemical features (**not anatomic location**) as **proximal-type (moderate - abundant mucinous and/or ciliated cells,** negative or weak TTF1 in luminal cells) or **distal-type (containing scant / absent mucinous and ciliated cells,** positive TTF1 in luminal cells). They are generally all peripheral in peribronchiolar sites.
- **Aim**: to evaluate whether PSCN-UMP represent a distinct entity or are a specific variant of BA and what is the relationship to SCC.

Methods: Compared the morphology, immunophenotype, and molecular profile of 10 PSCN-UMPs and 6 proximal-BAs (classic CMPTs) archives (2015–2020) of six institutions. *Why not compare with distal type which don't have mucinous or ciliated cells*

Results:
- Original diagnoses of 10 PSCN-UMPs included squamous cell carcinoma (*n* = 2), atypical squamous cell hyperplasia (*n* = 2), squamous papilloma (*n* = 3), bronchiolar adenoma (*n* = 1), and peribronchiolar squamous cell metaplasia (*n* = 2)\
- Clinicopathologic characteristics and driver mutations of PSCN-UMPs and BAs summarized in Table 1.
- PSCN-UMP - 10/10 females, median age 68 years (range: 49–76 years), nonsmokers average tumour size of 14 mm (range: 8–21 mm).
- BA - 5/6 females, average age 65 years (range: 50–74 years), nonsmokers, average tumour size of 17.5 mm (range: 10–32 mm)
- CT: All were peripheral well-defined solid / mixed solid/ground-glass nodules stable or grew very slowly.
- PSCN-UMPs
  - Biphasic cellular components, with surface luminal cells being reactive type II pneumocytes – no ciliated or mucinous cells
  - Basal layer - relatively bland non-keratinised stratified squamous cells, with hyperplastic squamous cells aligned between the surface pneumocytes and the alveolar basement membrane
  - Squamous cells - lepidic, inverted papillary / solid nested patterns in different proportions with transition between the different patterns
  - Squamous tufts were usually discontinuously extended along the alveolar wall into the surrounding air spaces sometimes filling them without obvious hyperplastic pneumocytes being present
  - Elastic stain confirmed there was no destruction of alveoli and the nests were confined within the retained alveolar architecture

- o Entrapped luminal cells -positive for TTF1, Napsin A, and CK7
- o TTF-1 clone used -8G7G3/1 (more specific than SPT24 and SP141)
- o Basal squamous cells were positive for p40, p63, and CK5/6
- o Squamous hyperplastic component co-expressed TTF1 and squamous markers
- o low proliferative activity
- o *EGFR* exon 20 insertions commonest (4/10)
- BAs
  - o typical morphology and immunophenotype of proximal bronchiolar pseudostratified columnar epithelium
  - o variable amount of mucinous and ciliated cells with a continuous layer of basal cells.
  - o Discontinuous skipping growth and spreading of micropapillary cellular tufts into adjacent alveolar spaces were observed in two cases
  - o Compared with PSCN-UMPs, the continuous basal cell layer rather than the stratified squamous epithelium was positive for p40 and CK5/6, with weak TTF1 staining in the basal cells and heterogenous positivity in the luminal cells.
  - o *KRAS* mutation, *BRAF* mutation, and *ERC1::RET* fusion were detected, no EGFR mutations.

Conclusions:
- PSCN-UMP is diffusely composed of well-differentiated squamous epithelium accompanied by non-neoplastic reactive pneumocytes, which is morphologically different from proximal-BAs.
- GP/MP arise in the **central airway**, PSCN-UMPs arise in the peripheral airway and their overt squamous differentiation with entrapped pneumocytes, and distinct genetic alterations (recurrent *EGFR* exon 20 insertions) of PSCN-UMPs are significantly different
- *BRAF*, *KRAS*, *EGFR*, *ALK*, and other lung cancer-related driver gene alterations are common in BA, whereas the most common *EGFR* mutations identified in BAs are rare *EGFR* exon 19 deletions, variants distinct from common sensitizing exon 19 deletions in lung adenocarcinoma
- "There is little overlap in morphologic, immunophenotype, and genetic features of the two neoplasms; therefore, the so-called PSCN-UMP is probably not a variant of BAs with overt squamous metaplasia". *However didn't compare with the 'distal' type BAs and the authors themselves mention that EGFR exon 20 insertions in two distal-type BAs have been identified previously (authors state in discussion that they could not compare to distal type BAs "due to lack of residual samples").*
- Comparison with peripheral-type squamous cell carcinoma (SCC)- destructive invasion, mitotic activity, desmoplastic response and necrosis.
  - o increasing in recent years
  - o Rarely, some SCCs developed in nonsmokers may harbour driver mutations such as the *EGFR* mutation (*EGFR* exon 20 insertions – 4 cases and *ALK* rearrangement
  - o peripheral SCC can display a lepidic growth pattern and present as ground glass nodules on chest CT
  - o The possibility of PSCN-UMP as a specific precursor lesion of peripheral SCC cannot be excluded.
- Limitations: small sample size due to the rarity of this entity and only archival samples were available for analysis.

Take home message: Authors made the statement: "A rare subset of BAs also displays marked basal cell hyperplasia and focal squamous metaplasia" - this description overlaps with the tumor described here as being a unique tumor. The jury is still out as to whether his peripheral pulmonary squamous cell neoplasm comprising the proliferation of bland squamous cells with entrapped hyperplastic pneumocytes is a unique tumor or may be a variant of BA. Coexpression of TTF1 and p40 and recurrent *EGFR* exon 20 insertions are apparently unique characteristic features but more cases need to be examined to see if this holds up.

4. Teng X, et. al., Bronchiolar adenoma with squamous metaplasia: a distinct phenotype. Histopathology 2023; 83: 193-201. https://doi.org/10.1111/his.14907

Background: The bronchiolar adenoma (BA), is a benign lung tumour characterised by nodular proliferation of bilayered bronchiolar-type epithelium with a continuous layer of basal cells. The authors identified a BA that had undergone squamous metaplasia and their **aim** was to fully characterize what they believe is a novel variant of BA

Methods: 5 cases from (from four patients) 2012 to 2019 underwent clinicopathologic, morphologic, IHC and molecular characterization.

Results:

- All female, mean age was 50 (ranging from 42 to 56) years. All patients were asymptomatic. A chest CT scan revealed solitary relatively well-circumscribed peripheral lesions adjacent to the bronchovascular bundle in all patients.
- Two cellular components, a surface of bilayered bronchiolar-type cells containing a continuous layer of basal cells and inner "stroma" comprised of sheets of spindle-oval and polygonal cells which represent squamous metaplasia
- All five cases had cilia on the surface epithelium, while mucinous cells were absent. A continuous layer of basal cells was detected subjacent to the columnar surface cells. Columnar epithelium was diffusely positive for TTF-1and Napsin A but negative for P40, P63, and CK5/6
- The stroma consisted of a sheet-like proliferation of relatively uniform, spindle-oval, and polygonal cells (squamous metaplasia) with no cytologic atypia, increased mitotic activity, or necrosis. Basal and squamous metaplastic cells positive for P40, P63, and CK5/6 (and negative for TTF-1). The Ki-67 proliferation index < 5%
- Mimicked the papillary pattern of round cells of pulmonary sclerosing pneumocytoma. However the stromal cells of PSP are positive with TTF-1. Molecular – 5/5 contained the *BRAF V600E* (c.1799T>A) mutation. Both basal cell layers and squamous metaplastic cells were positive for *BRAF V600E* staining in 3/5

Conclusion: Authors state that BASM is a distinct type of BA.

Take Home Message: Not convinced that this is a distinct variant of BA. Apart from the squamous metaplasia appears identical. Important to recognise this a benign tumor so as not to confuse with PSP or squamous cell carcinoma

Articles for Notation -Neoplastic

Butuo L, et al., Outcome-Supervised Deep Learning on Pathologic Whole Slide Images for Survival Prediction of Immunotherapy in Patients with Non-Small Cell Lung Cancer. Mod Pathol 2023, 36 (8), 100208. https://doi.org/10.1016/j.modpat.2023.100208

Summary: Approximately 60% of patients with NSCLC suffer from recurrence and metastasis after PD-(L)1 inhibitor treatment. **Aim**: To accurately predict the response to PD-(L)1inhibitors, using a deep learning model using a Vision Transformer (ViT) network based on H&E - stained specimens of patients with NSCLC. A total of 291 WSIs of H&E-stained histologic specimens from 198 patients in one centre and 62 WSIs from 30 patients in a second centre were included in the model training and validation. Patients had advanced NSCLC and were receiving PDL1 inhibitors as first or second line treatment. Primary endpoint was PFS of PDL1 inhibitor treatment and the patients were divided into 4 groups depending on PFS. The precision rate was 92.6% and the recall rate was 86%, superior to prediction by PDL-1 expression

Take home message: Promising model that may allow prediction of which patients will benefit from PDL-1 treatment but needs validation on larger cohorts. Although the authors stated the model was superior to that based on PDL1 expression but no data were supplied about the staining and scoring of PDL-1 therefore this last conclusion is questionable.

Rong R, et al., A Deep Learning Approach for Histology-Based Nucleus Segmentation and Tumor Microenvironment Characterization. Mod Pathol 2023, 100196. https://doi.org/10.1016/j.modpat.2023.100196

Microscopic examination of pathology slides is essential to disease diagnosis and biomedical research. However, traditional manual examination of tissue slides is laborious and subjective. Tumor whole-slide image (WSI) scanning is becoming part of routine clinical procedures and produces massive data that capture tumor histologic details at high resolution. Furthermore, the rapid development of deep learning algorithms has significantly increased the efficiency and accuracy of pathology image analysis. In light of this progress, digital pathology is fast becoming a powerful tool to assist pathologists. Studying tumor tissue and its surrounding microenvironment provides critical insight into tumor initiation, progression, metastasis, and potential therapeutic targets. Nucleus segmentation and classification are critical to pathology image analysis, especially in characterizing and quantifying the tumor microenvironment (TME). Computational algorithms have been developed for nucleus segmentation and TME quantification within image patches. However, existing algorithms are computationally intensive and time consuming for WSI analysis. This study presents Histology-based Detection using Yolo (HD-Yolo), a new method that significantly accelerates nucleus segmentation and TME quantification. We demonstrate that HD-Yolo outperforms existing WSI analysis methods in nucleus detection, classification accuracy, and computation time. We validated the advantages of the system on 3 different tissue types: lung cancer, liver cancer, and breast cancer. For breast cancer, nucleus features by HD-Yolo were more prognostically significant than both the estrogen receptor status by immunohistochemistry and the progesterone receptor status by immunohistochemistry. The WSI analysis pipeline and a real-time nucleus segmentation viewer are available at https://github.com/impromptuRong/hd_wsi.

Grosjean v, et. al, Hyalinizing clear cell carcinoma of the lung with EWSR1::CREM fusion. Histopath 2023, 83, 333-337. DOI: 10.1111/his.14942.

Summary: Case report of a 64-y woman, non-smoker with this tumor found incidentally; it was resected and at frozen section called carcinoma with a solid architecture. To date <15 described in the literature, all endobronchial, F>M and all have had EWSR1-ATF1 fusions apart from one case with EWSR1-CREM fusion. As expected, it stained similarly to a squamous cell carcinoma with p40 and CK5/6 positive and TTF-1 negative. Because this was a non-smoker, it was sent for molecular testing which prompted the correct diagnosis. This case is the second one with a EWSR1-CREM fusion.

Take Home Message: A very rare tumor but should be borne in mind when dealing with a central tumor that looks like SCC, especially if the patient is a non-smoker. The mitotic rate in the hyalinizing clear cell carcinoma is low to nil and has no necrosis.

Shen S, et al., Large-Scale Exome-Wide Association Study Identifies Novel Germline Mutations in Lung Cancer. Am J Respir Crit Care Med 2023, 208 (3), 280-289.
https://doi.org/10.1164/rccm.202212-2199OC
Rationale: Genome-wide association studies have identified common variants of lung cancer. However, the contribution of rare exome-wide variants, especially protein-coding variants, to cancers remains largely unexplored.
Objectives: To evaluate the role of human exomes in genetic predisposition to lung cancer.

Methods: comprehensively evaluated the exome-wide genetic variants of lung cancer in five independent cohorts - 30,312patients and 652,902 control subjects. Whole-exome sequencing (WES) determines the entire DNA sequence, not just known variants (as in SNP array-based studies), so it can find rare mutations that SNP-based genome-wide association studies may miss

Results: Systematically analyzed 216,739 single-nucleotide variants in the human exome. The loss-of-function variants had the most significant effects on lung cancer risk. The study identified eight variants that were more common in patients who developed lung cancer. Two were associated with smoking status, and the remaining six were associated with lung cancer independent of tobacco use. Four chromosomal regions previously associated with lung cancer risk were identified: TERT, MHC, CHRNA5, and CYP2A6 and four novel variants. The potential pathogenic relevance of the novel variants was demonstrated with RNA sequencing that expression levels of isoform gene variants differed significantly between paired tumor and normal lung tissue. The identified novel variants included two missense variants (TET3 and POT1) and two synonymous variants (TMEM173 and ATRN).

Limitations. The low rate of lung cancer and a relatively young mean age (56 yr) in the discovery cohort; this was almost entirely a study of persons of European ancestry.

Take Home Message: This paper is invaluable aid to helping to understand the biological underpinning's of genetics of lung cancer and should facilitate subsequent target analyses of specific exposures and genes.

Accompanying Editorial: Arenberg D. Lung Cancer Risk and Delivering on the Promise of the Genomic Era. Am J Resp Crit Care Med 2023, 208 (3), 226-228.
https://doi.org/10.1164/rccm.202306-1000ED

Eyerer FIR, et al., Getting Your Laboratory on Track with Neurotrophic Receptor Tyrosine Kinase. Arch Pathol Lab Med 2023, 147, 872-884. doi: 10.5858/arpa.2022-0042-CP.

Background: Neurotrophic receptor tyrosine kinase (NTRK) fusion testing has both diagnostic and therapeutic implications for patient care. With 2 tumor-agnostic US FDA–approved tropomyosin receptor kinase (TRK) inhibitors, testing is increasingly used for therapeutic decision making. Since the prevalence of NTRK fusions in the most common tumors (lung, breast, colorectal, prostate) is low, a cost effective screening method is necessary to identify the rare cases that would benefit from targeted therapy. However, the testing landscape for NTRK fusions is complex, and optimal testing depends on the clinicopathologic scenario.

Objective: A literature search to compare different NTRK testing methods to help pathologists understand test features and performance characteristics and make appropriate selections for NTRK fusion detection for their laboratory and individual patient specimens.

- Staining patterns may show different cellular localization depending on the fusion,; it is likely the fusion partner, not the NTRK gene, that determines the staining pattern.
- Sensitivity and specificity of pan-TRK IHC vary related to the tumor type tested
- IHC will detect expression of wild-type TRK due to either amplification or high tissue-specific expression.
- Physiologically normal TRKs may be expressed in tumors, particularly sarcomas with neural or smooth muscle differentiation, leading to false-positive staining and decreased specificity.
- RNA is ideal for the identification of fusions as it obviates the need for intronic coverage, has high analytic sensitivity for all breakpoints for targeted fusions, and may detect fusions in the setting of a low tumor percentage if the fusion is overexpressed.

Conclusions: As standard of care in some tumor types, next-generation sequencing (NGS) panel testing is a cost effective and reliable way to detect a broad range of NTRK fusions. The design of the panel and use of DNA or RNA will affect performance characteristics. Pan-TRK IHC may be used as a rapid, less expensive screen in cases that will not undergo routine NGS testing, or on specimens unsuitable for NGS testing. FISH may be appropriate for low-tumor-content specimens that are unsuitable for NGS testing. Quantitative RT-PCR is best suited for monitoring low-level disease of a specific, previously identified target.

Take Home Message: A relatively clear presentation of the complexities of NTRK testing which should help laboratories develop their own algorithm depending on the size of the lab, population and resources.

Weissferdt A, et al., Primary epithelioid hemangioendotheliomas and angiosarcomas of the pleura: a clinicopathological and immunohistochemical analysis of 13 cases. Hum Path 2023, 138, 68-75. https://doi.org/10.1016/j.humpath.2023.06.010

Summary: Case series of primary epithelioid hemangioendotheliomas (EHE, n = 8) and epithelioid angiosarcomas (EA, n = 5) of the pleura. 7/13 men, mean age 47 (34-65). Diagnostic imaging - either diffuse pleural thickening or pleural nodules. Open surgical biopsies were obtained in all cases. EHE all had mild to moderate atypica and low mitotic activity whilst EA had areas of necrosis, hemorrhage, marked cytologic atypia and mitotic activity. Both tumors were positive for CD31, CD34 and ERG with only EHE being positive for CAMTA1. Clinical follow-up obtained in eleven cases showed that all patients had died within 30 months post diagnosis, therefore the "low-grade" appearance of EHE belies a uniformly bad prognosis, at least in the pleura. The authors suggest that primary pleural origin of these tumors appears may indicate a more aggressive clinical behavior.

Take Home Message: Although these tumors are relatively rare, they can mimic mesothelioma clinically and radiologically. Important to remember that they can also stain with pan cytokeratins

Suster D, et al., Thymic Parenchymal Hyperplasia. Mod Path 2023, 36 (8), 100207.

Summary: Two types of hyperplasia of the thymus:1) Thymic hyperplasia: lymphoid follicular hyperplasia associated with autoimmune disorders. This is due to increased migration of B cells into the perivascular spaces, which are located adjacent but outside of the thymic epithelial network. 2)True thymic *parenchymal* hyperplasia: extremely rare, especially in adults and may give rise to difficulties in diagnosis. Characterized by the expansion of the thymic epithelial network by immature thymocytes. The authors prefer the term intraparenchymal as the increase is within the thymic parenchyma unlike thymic hyperplasia which is extraparenchymal.

44 patients (38 F)) aged 7 months to 64years (mean, 36 years) with thymic parenchymal hyperplasia were studied. 18 presented with symptoms of chest discomfort or shortness of breath; in 20 patients, the lesions were discovered incidentally. All patients were treated with complete surgical excision. The tumors measured from 3.5 to 24 cm (mean, 10.46 cm).

Histology: lobules of thymic tissue displaying well-developed corticomedullary architecture, with scattered Hassall corpuscles separated by mature adipose tissue of varying amounts, and bounded by a thin fibrous capsule. In two cases the cortical and subcortical areas displayed a prominent starry-sky appearance due to numerous scattered tingible body macrophages. No lymphoid follicular hyperplasia, cytologic atypia, or confluence of the lobules. IHC stained similarly as normal thymus in childhood and adolescence. The commonest diagnosis initially was type B1 thymoma. However the latter shows distortion of the normal thymic architecture with enlarged confluent thymic lobules separate by thick fibrous bands and with a thick capsule; the medullary zones are not well preserved and there may be dilated perivascular spaces which are not a feature of hyperplasia. In type B1 thymoma, epithelial cells are scattered and scanty, whilst in hyperplasia the keratin-positive epithelial cells showed a striking condensation at the periphery of the lobule forming a meshwork. See **Table 2** in the paper.

Clinical follow-up in 26 cases showed that all patients were alive and well between 5 and 15 years after diagnosis (mean, 9 years).

Take Home Message: "True" thymic parenchymal/intraparenchymal hyperplasia can present like a malignancy with some cases even showing a mass was wrapped around the superior vena cava and innominate vein and showing rapid increase in size. As well as volume and weight of the gland in defining thymic parenchymal hyperplasia, the age of the patient should also be factored in. The differentiation between thymic parenchymal hyperplasia and thymoma can be difficult in small core biopsies but if dilated perivascular spaces are seen this points to thymoma.

Articles for Notation -Non-neoplastic

Vanderbeke L, et al., A Pathology-based Case Series of Influenza- and COVID-19-associated Pulmonary Aspergillosis – The Proof is in the Tissue. Am J Respir Crit Care Med 2023, 208 (3), 301-311. DOI:10.1164/rccm.202208-1570OC.

Comment on this article: Albrich WC, et al., Viral-associated Pulmonary Aspergillosis: Have We Finally Overcome the Debate of Colonization versus Infection? Am J Respir Crit Care Med, 208 (3), 230-231. DOI:10.1164/rccm.202306-1022ED.

Background: Invasive pulmonary aspergillosis is a frequent coinfection in severe COVID-19, "CAPA" similar to influenza. Invasiveness debated:

-low sensitivity of serum mycological markers

- reports on survival despite withholding antifungal treatment

- scarce evidence of proven disease upon histopathological exam

Objectives: To investigate the invasive nature of pulmonary aspergillosis in histology specimens of influenza and COVID-19 ICU fatalities in a tertiary care center.

Methods: Adult ICU patients with PCR-proven influenza/COVID-19 respiratory failure underwent postmortem examination and/or tracheobronchial biopsy during ICU admission from September 2009 until June 2021. Diagnosis of probable/proven viral-associated pulmonary aspergillosis (VAPA) was made based on international Society COVID-19–associated pulmonary aspergillosis consensus criteria. All respiratory tissues were independently reviewed by two experienced pathologists.

Results:

- 44 patients with influenza (n = 21) and Covid-19 (n = 23) underwent autopsy.
- 6 influenza-associated (VAPA) and 6 COVID-19–associated (CAPA) pulmonary aspergillosis diagnoses
- Therefore prevalence of proven VAPA of at least 12% and CAPA in at least 8% (n = 6/74) among all ICU influenza and COVID-19 fatalities
- Likely under-represented as autopsy rate overall 35%
- Anti-fungal treatment had been started in 11/12
- Fungal disease - a missed diagnosis upon autopsy in 1/12
- BAL galactomannan testing showed the highest sensitivity for VAPA diagnosis.
- Impeded fungal growth (sparsely dispersed, fragmented hyphae found in acutely inflamed and/or necrotic lung) was the predominant histologic pattern of pulmonary aspergillosis.
- Fungal tracheobronchitis histologically indistinguishable in influenza (n= 3) and COVID-19 (n= 3) cases- macroscopically more extensive at bronchoscopy in influenza setting.
- Previous antifungal therapy might have been a cause of false-negative autopsy results, as patients with probable unconfirmed VAPA received for longer than those with proven VAPA

Conclusions:

- A proven invasive pulmonary aspergillosis diagnosis was found regularly and with a similar histological pattern in influenza and in COVID-19 ICU case fatalities
- The debate as to whether positive culture represents invasive vs colonization by apergillus - more evidence that it represents invasion in ICU patients

Take Home Message:

A high awareness of invasive aspergillus in patients with viral infection is needed and a diligent search for fungal hyphae that may be distorted and scanty is needed, especially in a necrotic and/or inflamed background.

Schwab C, et al., Lethality of SARS-CoV-2 infection – a comparative autopsy study focusing on COVID-19 development and virus variants. Histopathology 2023, 83, 242-251. DOI: 10.1111/his.14931.

Background:

1. Different SARS-CoV-2 variants are driving various waves of infection of the corona pandemic.
2. Aim: to address the effect of the different variants evolving during the pandemic on fatal outcomes.

Methods and results:

- Autopsies on 117 people who died of a (*?with*) SARS-CoV-2 infection and the findings were interpreted in clinical and pathophysiological contexts.
- COVID-19-related lung injury was significantly less common (50 vs 80–100%) and less severe in cases infected by omicron variants compared to precedent variants($P<0.05$).
- COVID-19 was less often the leading cause of death following omicron infection.
- Extrapulmonary manifestations of COVID-19 did not contribute to death in this cohort.
- Lethal COVID-19 may occur after complete SARS-CoV-2 vaccination, but not within 4 months of the last injection in completely vaccinated people.
- Reinfection was not the cause of death in any of the autopsies of this cohort.

Conclusion: Compared to previous variants, infection with an omicron variant affected the lungs less frequently and resulted in less severe lung disease.

Frank AJ, et al., Case 24-2023: A 43-Year-Old Man with a Pulmonary Nodule. N Engle J Med 2023, 389, 550-558. DOI: 10.1056/NEJMcpc2300911.

Summary:

- A 43-year-old man who resided in Panama- incidentally found pulmonary nodule in RML 12 x 13 mm
- Endobronchial ultrasonography with transbronchial needle aspiration and cervical mediastinoscopy
- Necrotizing granulomatous inflammation
- GMS: Narrow-based budding yeasts, round to ovid, 3-4 microns diameter, no capsule
- Serology positive for Histoplasma and negative for Cryptococcus
- Histoplasmosis is a very common cause of pulmonary nodules
- Unlikely to grow in culture (but no tissue was sent for culture)

Korzhuk A, et al., Sepsis in Immunocompromised Host and Hematologic Complication. Chest 2023, 164 (2) e47-e55. DOI: https://doi.org/10.1016/j.chest.2023.03.015.

Summary:

- 43-year-old Puerto Rican man with a kidney transplant being treated for acute transplant rejection presented with 2 weeks of flu-like symptoms, nausea, and vomiting
- CT scans - opacities as possibly early tree-in-bud opacities
- Discharged with doxycycline for nonspecific pneumonia
- He returned to the hospital 4 days later with fevers, chills, sweating, dyspnea, and diarrhea and purple maculopapular rash on his forehead and back
- CT scan - diffuse multifocal pneumonia new from previous imaging, expansile lytic lesions on multiple ribs and right iliac bone, moderate splenomegaly, mass-like area in right native kidney
- Bronchoscopy images showed numerous papular lesions in multiple locations in all visible airways

- Skin biopsy: sheets of histiocytes within the superficial dermis that extended into the overlying squamous epithelium; within histiocytes numerous intracellular encapsulated fungal microorganisms
- BMBx showed normal cellularity with trilineage hematopoiesis and hemophagocytosis
- BAL, endobronchial and transbronchial biopsy: "encapsulated" fungal microorganisms
- Immunosuppression history, splenomegaly, two lineage cytopenias, ferritin, triglyceride, fibrinogen and aspartate aminotransferase trended levels summated to an hemophagocytic lymphohistiocytosis (HLH)-probability calculator (H-score) of 208
- Diagnosis: Histoplasmosis-induced hemophagocytic lymphohistiocytosis (HLH) likely related to H. duboisii *(?species)* contracted whilst visiting Puerto Rico at the time of his increased immunosuppression (*but could be reactivation of latent infection too*)
- IV immunoglobulin and high-dose dexamethasone were given with good clinical response.

Critique: Authors described the organisms as being encapsulate. Histoplasma is **not** encapsulated as such but has a cell wall that is highlighted with GMS and PAS stains
The authors assumed the species was H. duboisii because of skin and bone lesions, but this does not distinguish the species in the face of an immunocompromised patient with disseminated Histoplasmosis,