

Journal Club
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Articles for Discussion

1. Pulmonary manifestations of GATA2 deficiency. Marciano BE, Olivier KN, Folio LR, Zerbe CS, Hsu AP, Freeman AF, Filie AC, Spinner MA, Sanchez LA, Lovell JP, Parta M, Cuellar-Rodriguez JM, Hickstein DD, Holland SM. *Chest*. 2021;160(4):1350-1359.

Background: GATA2 deficiency (G2D) is a recently described primary immunodeficiency syndrome that affects hematopoiesis, lymphatics, and immunity. It's transmitted as an autosomal dominant or sporadic disease and often recognized by severe or recurrent infections, lymphedema, monocytopenia, dendritic cell cytopenias, myelodysplastic syndromes and AML. GATA2 is a "zinc finger transcription factor essential for differentiation of endothelial and immature hematopoietic cells". It's also involved in promoting phagocytosis by alveolar macs. the current study describes pulmonary manifestations of G2D in a large cohort followed up at a single center (NIH).

Methods: A retrospective review of medical records, imaging, pathology and PFTs in 124 pts for patients (95 probands, 29 ascertained through screening) with mutation-proven G2D between '1992 and 2020.

Results: Age:8 to 86, median in the mid-30s. Male/female ratio approximately 3:1.

- One of the most common modes of presentation was disseminated or pulmonary mycobacterial infections occurring in late childhood or adulthood. Others had recurrent bronchitis or multiple pneumonias. A few had fungal infections.
- Pathology samples (BAL: 38 probands; lung biopsies, type unstated: 5 probands; or autopsy, 7 probands) available.
- PAP in 11/95, with only 1/11 with anti-GM-CSF antibodies (8 biopsy based).
- BAL showed lymphocytic (23%), monocytic (40%) or neutrophilic (38%) predominances.
- Biopsies showed chronic lymphohistiocytic inflammation with scattered eos; no granulomas. Interestingly, lung specimens demonstrated "an abundance of alveolar macrophages" despite the absence of peripheral blood monocytes.
- Radiology in absence of active infections included apical predominant reticulations, central vascular prominence, and paraseptal emphysema, sometimes extensive. Non-specific bronchiectasis was also identified in 10% of probands.

Treatment and response discussed in ms.

Discussion: Several mechanisms may be at work including abnormal alveolar macs, impaired pulmonary lymphatic circulation, impaired phagocytosis, and possibly the lack of new monocytes repopulating the pulmonary compartment.

Comment: Disease to suggest in the setting of alveolar proteinosis and in the setting of atypical mycobacterial infections that don't fit the usual categories.

2. Sarcoidosis: an occupational disease? Oliver LC, Zarnke AM. Chest. 2021;160(4):1360-7.

Background: In the last 10-20 years epidemiologic studies and case reports have provided evidence of causal associations between occupational exposure to specific agents and sarcoidosis. This article is a review of these recent advances.

Methods: Review of the recent literature with a focus on those exposures for which there is greater consistency and “strength of association”.

Results: Table 1 shows reported associations. The authors believe data most compelling for a strong association with silica and silicates, World Trade Center collapse, and metals.

Occupation/Industry	Exposure	
	Organic	Inorganic
Agricultural work	Fungi, dusts	Silicates, insecticides
Construction work		Silica, concrete, metals
Firefighting/EMS work		WTC dust, other dusts, fumes
Foundry work		Silica, metal dusts, metal fumes
Glass wool, rock wool work		MMMF, silica, Al, Ti
Lumber industry		Wood dust
Metal industry		Metal dusts, metal fumes
Mining		Silica, silicates, other dusts, fumes
Office work	Fungi, other microbes, musty odors	
Transportation		Metal dusts, other dusts
Tunnel construction	Fungi, musty odors	Silica, other dusts, fumes

Al=aluminum; EMS=emergency medical services; MMMF=man-made mineral fibers; Ti=titanium; WTC=World Trade Center

1. Silica and Silicates: data comes from case reports, case controlled epidemiologic studies.
2. World Trade Center dust: major components, limestone, gypsum, bassanite, and crystalline silica. Also relatively high concentrations of manganese, AL, barium, and Ti from building construction materials and paint. Smaller amounts of Cr, Pb, zinc, and other organic compounds. A few relevant studies include
 - a. Caplan-Shaw et al (J Occup Environ Med 2011;53:981-991) examined lung tissue from WTC exposed residents and local workers with abnormal imaging and PFTs. Lung tissue from 5/12 revealed aluminum silicate, titanium, and talc. Silica was found in 4/5 and steel (FeCrNi) in three.
 - b. A couple of studies have shown Bx-confirmed sarcoid like granulomatous lung disease increased significantly in NYC firefighters compared to the 15 years prior.
3. Metal dusts: metals can cause an antigen specific granulomatous immune response and a “nonspecific innate immune response” triggered by oxidant injury. Much of the data for this association comes from studies using the MELISA lymphocyte proliferation tests with metals other than beryllium e.g Ti, Ni, Cr, Mercury, and palladium.

Discussion: The authors propose that when sarcoidosis is suspected, a detailed occupational and environmental history should be obtained, and that sarcoidosis should no longer be considered idiopathic until this has been done. They also suggest that the use of lymphocyte proliferation tests be used as diagnostic tools in larger studies for validation.

Comment: Pay attention to the history in sarcoid cases! It might be relevant. I guess what we don't know if avoidance of the offending agent will affect the course of sarcoidosis.

3. Grading in lung adenocarcinoma: another new normal (Editorial). Nicholson AG, Moreira AL, Mino-Kenudson M, Popat S. J Thorac Oncol. 2021;16(10):1601-4.

Background: Editorial for paper 4 and 5.

Table 1. Comparison of Proposed Grading Systems

Grade	IASLC	Sica et al (Two predominant patterns)	Architectural
Low (grade 1)	Lepidic predominant with <20% of a high-grade pattern(s)	Lepidic with acinar or papillary	Lepidic predominant
Intermediate (grade 2)	Acinar or papillary predominant with <20% of a high-grade pattern(s)	Pure acinar or papillary OR mixed acinar and papillary OR lepidic with solid or micropapillary	Acinar or papillary predominant
High (grade 3)	Any tumor with ≥20% of a high-grade pattern(s)	Acinar or papillary with micropapillary or solid OR pure solid or micropapillary OR mixed solid and micropapillary	Solid, micropapillary, cribriform (CGP) predominant

CGP=complex glandular pattern; IASLC=International Association for the Study of Lung Cancer

Discussion: The authors of this editorial ask why grade cancers at all if it is only used to drive prognostication and not used to plan for clinical care as is now the case for prostate and breast. They propose that future trials incorporate grade as well as stage. This conclusion is in part supported by observations by Deng et al (see below) who showed improved prognosis with adjuvant chemotherapy in patients with stages Ib to III with high grade adenocarcinoma on the basis of the IASLC grading system. They also recognize that additional work needs to be done on other tumor types including squamous carcinomas and mucinous carcinomas.

The authors are relatively pleased with the overall interobserver agreement rate between these and other recent studies which range from 0.61 to 0.94.

4. Validation of the novel international association for the study of lung cancer grading system for invasive pulmonary adenocarcinoma and association with common driver mutations. Deng C, Zheng Q, Zhang Y, Jin Y, Shen X, Nie X, Fu F, Ma X, Ma Z, Wen Z, Wang S, Li Y, Chen H. *J Thorac Oncol.* 2021;16(10):1684-93.

Background: The authors aimed to validate the use of the IASLC classification for adenocarcinoma in Chinese patients and correlate the grading system with common driver mutations and response to adjuvant chemotherapy.

Methods: 950 patients with invasive ADC (stage I-III) from 2008-2016 were retrospectively analyzed and classified according to the proposed grading system. Tumor grading was correlated with genetic data, response to adjuvant chemotherapy, and patient outcomes. AIS, MIA and invasive mucinous adenocarcinomas excluded.

Results: Compared with the architectural pattern-based groups the IASLC grading system carried improved survival discrimination (AOC 0.768 for recurrence-free survival and 0.775 for overall survival).

- AOC was not further improved when lymphovascular invasion was incorporated.
- Kappa value for IASLC grading system higher than for the conventional architecturally based groups (0.633 versus 0.581). Most discrepant cases were attributed to observed differences in proportion of high-grade patterns particularly those close to the threshold of 20%.

EGFR mutations were correlated with moderately differentiated tumors. KRAS mutations and ALK fusions were more prevalent in higher grade tumors.

Incorporating EGFR mutation status into the grading system showed excellent survival discrimination ($P < 0.001$). In particular, patients with stage Ib to III with high-grade adenocarcinomas treated with adjuvant chemotherapy had improved prognosis.

Discussion: The IASLC system is a good discriminator for patient prognosis and should be part of a pathologic/genetic subtyping to improve prognostication. It may also support patient stratification for chemotherapy.

- Validation study of the International Association for the Study of Lung Cancer Histologic Grading System of invasive lung adenocarcinoma. Rokutan-Kurata M, Yoshizawa A, Ueno K, Nakajima N, Terada K, Hamaji M, Sonobe M, Menju T, Date H, Morita S, Haga H. *J Thorac Oncol.* 2021;16(10):1753-8.

Background: Three different grading schemes for nonmucinous adenocarcinoma had been proposed including the more recent one by the IASLC Pathology Committee, now listed in the new WHO Blue Book. The authors were particularly interested in the validation of the IASLC grading system in Asian patients.

Methods: The authors performed comprehensive histologic subtyping and graded them according to the architectural pattern system (ARCH), grading system based on two predominant patterns (Sica system) and the newer IASLC system. Concordance index, and receiver-operating characteristic curves were used to evaluate clinical utility of the systems for recurrence and death. Cases of AIS and MIA were excluded.

Results: Study cohort: 1002 patients with invasive nonmucinous adenocarcinoma (235 developed recurrent disease and 166 died).

Concordance index (C-index) and ROC. The table reflects the performance of each grading system for disease free survival and overall survival using the area on the area under the curve (AUC) of the time dependent ROC and C-Index at five years. Interested parties can read more about the statistical analysis.

They also evaluated the interobserver agreement using 100 randomly selected cases. The kappa for the IASLC grading system was 0.94 between two pathologists.

System	Recurrence		Death	
	CI	AOC	CI	AOC
IASLC	0.777	0.807	0.767	0.776
Arch	0.763	0.796	0.743	0.755
Sica	0.786	0.814	0.762	0.773

Discussion: The authors conclude that the IASLC model is acceptable as a prognostic indicator for both recurrence and death in their large Asian cohort but that the Sica system yielded nearly identical results. The authors acknowledge the limitation of the study which did not include invasive mucinous adenocarcinoma or minimally invasive carcinomas.

Comment: The ARCH system is really not that much different, seems to me, but the authors don't comment on that.

From Dr. Ping Yang one of our statisticians with a strong interest in lung cancer.

1. Straightforward comparison on all-stage, I concur with your conclusion; also, the difference between using IASLC and ARCH appears not clinically meaningful.

2. More meaningful (to me) is the superiority of Sica in Stage-I patients, particularly for DFS, which could be more clinically relevant considering adjuvant appropriate therapy; however, noted is the globally reduced CI and AUC measures.

3. Recognizing the purpose of this work was to compare the 3 grading systems, 2 major caveats shouldn't be ignored are as follows:

(1) The baseline models (in Table-2) seemed not built fully or most properly, which only included age and crude stage; other potential prognostic factors, some of which are listed in Table-1 were not used, and no reasons provided. A potential result would be the best-built clinical/baseline model performing at the same level as the 3 comparative models using alternative grading systems; in addition, some of these known factors may confound the effect of grading systems at varied magnitudes.

(2) As authors mentioned, the IMA group was excluded, a relative aggressive subtype may sway the current results in unpredictable directions.

Neoplastic Notations

1. Expression patterns for Bcl-2, EMA, β -catenin, E-cadherin, PAX8, and MIB1 in thymomas. Suster D, Miller JA, Pihan G, Mackinnon AC, Suster S. Mod Pathol. 2021;34:1831-8.

Background: Expression patterns of IHC have been extensively investigated in thymomas to assist in differential diagnosis. These authors selected six markers to determine their utility in the evaluation of the differential diagnosis for thymic neoplasms.

Methods: Thymomas were collected from Beth Israel and U Wisconsin from 1990-2016. Diagnoses of thymoma confirmed and included only examples of the major WHO thymoma categories. 130 cases identified and 4 were eliminated because of inadequate material in the TMA. Study set included 33 type A, 27 type AB, 20 type B1, 22 type B2 and 24 type B3. Only “pure” cases were used to reduce heterogeneity in the cores. Stains used were BCL2, EMA, β -catenin, e-cadherin, PAX8 and MIB1.

Results: Table 1. Results of immunohistochemical markers for E-cadherin, β -catenin, PAX8, EMA, bcl-2, and MIB-1 in 126 cases of thymomas

Type	E-CAD	β -CAT	PAX8	EMA	BCL-2	MIB-1
A	100%	97%	54.5%	9% ^a	90%	0.74%
AB	92%	100%	66.6%	37% ^a	88.8%	0.57%
B1	100%	100%	50%	0%	0%	1.60%
B2	45%	100%	40.9%	0%	0%	1.77%
B3	100%	100%	66.6%	20% ^b	4%	12.79%

E-CAD=E-cadherin; β -CAT = beta-catenin; CI = confidence intervals

^aPositivity only in spindle cells within fibrous septa

^bPositivity only in microscopic foci of squamous differentiation

- All cases- 100% strong p63 and AE1/3 reactivity.
- BCL2 (diffuse cytoplasmic staining) consistently positive in type A and AB (approximately 90%) while 100% of types B1-3 were negative. (Other studies, however, have not consistently shown this).
- E-cadherin and β -catenin were not useful in differential diagnosis.
- MIB-1 was highest in type B3 compared with other histologic types. See figure 4.
- EMA generally negative except for spindle cells in fibrous septa of types A and AB.
- PAX8 showed less consistent nuclear staining than p63 (55.7 vs 100%).

Discussion: The most significant finding was the use of BCL2 to highlight types A and AB (as opposed to type Bs). It’s surprising EMA proved to be useful due to its staining of the fibrous septa in type A and type AB while it failed to stain septa in type B1-3.

- Authors conclude β -catenin and E-cadherin not useful due to broad overlap and inconsistent expression across types.
- PAX8 if going to be used must be polyclonal antibody; but p63 may be better marker.
- MIB-1 may be helpful in separating B2 from B3 in this setting. CD45 may also be beneficial in showing a lower content of lymphocytes in type B3.

Comment: It might be worth giving these stains a try on your next challenging thymoma case.

2. Micronodular thymic carcinoma with lymphoid hyperplasia: relevance of immunohistochemistry with a small panel of antibodies for diagnosis: a RYTHMIC study. De Montpreville VT, Mansuet-Lupo A, Le Naoures C, Chalabreysse L, De Muret A, Hofman V, Roquette I, Piton N, Dubois R, Benitez JC, Girard N, Besse B, Marx A, Molina TJ. Virchows Archiv. 2021;479:741-6.

Background: Micronodular thymic carcinoma with lymphoid hyperplasia (MNTCLH) is a rare low aggressive form of thymic carcinoma. The main differential for this neoplasm is micronodular thymoma. The authors sought to use a panel of IHC to differentiate among these two entities.

Methods: A French group devoted to treatment of TET identified 6 MNTLCs among 1007 cases. 26 micronodular thymomas (MT) were also identified for comparison.

Results: The authors give a significant amount of clinical information on these cases (see paper, Table 1).

Micronodular thymic cancer

Tumor	AE1/3	p63	TdT	CD117	CD20	CD5	Glut1
MNTCLH n=6	+ epi cells	+ epi cells	- lymphs	+ epi	+ lymphs - epi	+ epi	+epi
MT n=26	?	?	+ lymphs	-	?	-	?

MNTCLH = micronodular thymic carcinoma with lymphoid hyperplasia

MT = micronodular thymoma

Epi = neoplastic epithelial cells

Discussion: The authors highlight some of the challenges in making the diagnosis of MNTCLH including:

- Overlooking mitotic activity and atypia
- CD5 not consistently positive in reported cases; CD117 also strongly positive
- GLUT1 reactivity may be helpful as it is reportedly negative in thymomas, especially in the micronodular type (although they don't actually give these results in their study)

Comment: I would say whenever you get an unusual thymoma share it with Dr. Roden!

3. Primary lung carcinoma in children and adolescents: clinical characteristics and outcome of 12 cases from the German registry for rare paediatric tumours (STEP). Voggel S, Abele M, Seitz C, Agaimy A, Vokuhl C, Dirksen U, Bier A, Flaadt T, Classen CF, Claviez A, Schneider DT, Brecht IB. *Lung Cancer*. 2021;160:66-72.

Background: Primary pediatric lung tumors represent < 0.1% of all childhood malignancies. The current study identified patients less than 18 years of age with primary lung malignancies from the German Childhood Cancer Registry.

Methods: 12 patients and adolescents were identified from 2009-2019. Patients with NUT carcinoma were excluded.

Results: Mucoepidermoid carcinoma: 83%

- 6 low-grade; MAML2 rearrangement present in 3/3
- Squamous carcinoma 2/12; both had advanced disease at diagnosis; 1 patient had oro-facial-digital syndrome type II (Mohr's syndrome); No patient had respiratory patholomatosis
- Adenocarcinoma 2/12
- Adenosquamous carcinoma 1/12

Discussion: MEC is the most common primary lung carcinoma in childhood consistent with other studies.

Comment: Perhaps somewhat surprising that neither of the adenocarcinomas were associated with pre-existing cystic lung lesions

4. Analysis of microRNA expression in liquid-based cytology samples may be useful for primary lung cancer diagnosis. Araki Y, Arihiro K, Yamaguchi K, Sakamoto S, Horimasu Y, Masuda T, Miyamoto S, Nakashima T, Iwamoto H, Fujitaka K, Hamada H, Hattori N. *Am J Clin Pathol*. 2021;156:644-52.

Background: MicroRNAs (miRs) are small noncoding RNAs consisting of about 22 nucleotides that play an important role in regulating gene expression. Frequently a diagnosis of carcinoma fails to be established after bronchoscopy and EBUS. The authors examine the value of looking for microRNAs extracted from liquid-based cytology.

Methods: Two data sets:

1. 18 primary lung cancer resections with paired tumor and normal used as controls.
2. Cytologic samples: 136 lavage fluid and washed device fluid samples. Ten samples excluded (metastatic carcinoma, inadequate samples).

RT-qPCRs was performed for miR-21, miR-31, miR-182, and miR-183, selected due to prior studies showing these might be promising biomarkers in lung cancer.

Expression of the four miRs was compared with pathologic diagnosis. Histology of surgical resections (31) and biopsy sample (50) were used to classify cases as either benign or malignant depending on what was identified, e.g., granuloma versus tumor.

There was no significant difference in miRs expression among lung cancer subtypes

Results:

Primary lung cancer versus noncancerous lung tissue (18 pairs):

Relative expression levels of 4 miRs in CA significantly higher than in adjacent noncancerous tissues ($P < .05$).

Cytologic samples:

4 miRs analyzed in 125 cytology samples: cancer cases 83, noncancer cases 42.

- Expression of all 4 miRs was significantly higher in patients with lung cancer than in those without lung cancer.
- “Among samples judged as benign or indeterminate, levels of these miRs were also significantly higher in patients with lung cancer than in those without lung cancer.” ?
I am not sure what the authors mean by this statement.

Discussion: The authors conclude that miR expression in liquid-based cytology samples may be helpful in diagnosing primary lung cancer.

Limitations:

- Many samples in which miR expression was undetectable
- Although one perhaps can identify a high likelihood of a patient having cancer based on elevated miRs in LBC, one still needs definitive tissue for diagnosis of tumor type and potentially molecular testing

Comment: Parts of the article I find unclear and a bit confusing. The authors do demonstrate that miRs can be extracted from LBC samples, but I am not sure how clinically relevant this will turn out to be.

5. Use of the idylla EGFR mutation test for variant detection in non-small cell lung cancer samples. Hawkins P, Stevenson T, Powari M. Am J Clin Pathol. 2021;156:653-60.

Background: The Idylla system is a fully automated platform designed to rapidly genotype formalin-fixed paraffin-embedded tissue samples for EGFR mutation. This retrospective study was designed to investigate the use of the assay for EGFR detection.

Methods: The Idylla and reference testing methods were compared using DNA preparations extracted previously from a variety of routine histologic and cytologic samples ($n = 33$). Sanger sequencing was a reference method.

Results: The Idylla test yielded valid results for all samples tested confirming variants identified by the Sanger method that lay within the Idylla target range.

No false positives.

Variant genotype reports were obtained within 150 minutes. Tables 1 and 2 list all of the mutations identified.

Discussion: The authors recognize that NGS has become the standard of practice but argue that rarely in patients with acute deterioration or in those developing a resistance mutation. The inherent delay NGS testing could be alleviated by the Idylla mutation test.

6. YAP1 protein expression has variant prognostic significance in small cell lung cancer (SCLC) stratified by histological subtypes. Wang X, Guo Y, Liu L, Wei J, Zhang J, Xie T, Dong J, Li J, Xing P, Yang L. Lung Cancer. 2021;160:166-74.

Background: Small cell lung cancer (SCLC) has been considered a homogeneous disease even though combined small cell carcinomas (C-SCLC) ranges from 2%-28% in various studies. Recently, it has been observed in some studies that C-SCLC have a lower response rate to chemotherapy compared to pure SCLC (p-SCLC).

Recently, 4 molecular phenotypes of small cell carcinoma have been identified based on YAP1 expression and the presence of achaete-scute family bHLH transcription factor 1 (ASCL1), neuronal differentiation 1 (NEUROD1) and POU class 2 homeobox 3 (POU2F3). The relevance and prognostic significance of YAP1 has not been studied.

YAP1 is a downstream nuclear effector of the inactivated Hippo signal pathway essential for regulating cell proliferation, apoptosis, stem/progenitor cell expansion and organ growth; it has also been identified as a tumor marker associated with sensitivity to drugs in various cancers including NSCLC and SCLC. This study compared the expression of YAP1 in SCLC and C-SCLC (the small cell components) to explore its prognostic correlation.

Methods: 297 patients who had “surgery” and diagnosed as SCLC between 2005-2016 were included: 251 P-SCLC and 46 C-SCLC.

YAP1 quantified by an H-score: intensity 0-3 resulting in 4 grades: and % distribution: score 0-9, negative; score 10-49, 1+; score 50-149, 2+; score 150-300, 3+. Other stains also performed included P63, P40, TTF-1, Napsin, chromogranin, CD56 and synaptophysin. Treatment and follow-up data also collected.

Results: YAP1 staining is nuclear.

Clinical parameters were well balanced between the two subtypes.

YAP1 expression significantly higher in C-SCLCs than P-SCLCs (52.2 vs 29.1% , p=0.004).

Expression of YAP1 was associated with worse overall survival (39% vs 74.9%) and with independent risk factor for overall survival in C-SCLC. Expression of the YAP1 showed no prognostic impact in P-SCLC.

Discussion: Expression of the YAP1 in small cell components of C-SCLC significantly higher than P-SCLC and was an unfavorable predictor for overall survival in this group.

This observation may lead to potential differential targetable oncogenic pathways for future treatment.

Comment: A remarkable number of surgically resected small cell lung carcinomas in this study; it would be interesting to know how well this works on smaller samples.

7. CD163-positive cancer cells are a predictor of a worse clinical course in lung adenocarcinoma. Matsubara E, Komohara Y, Shinchi Y, Mito R, Fujiwara Y, Ikeda K, Shima T, Shimoda M, Kanai Y, Sakagami T, Suzuki M. *Pathol Int.* 2021;71:666-73.

Background: CD163 is a 130kDa transmembrane protein involved in hemoglobin clearance as a scavenger receptor for the hemoglobin-haptoglobin complex. It is expressed on monocytes and macrophages but it's also recently been identified in cancer cells including bladder, breast, rectal, and renal cell where it has been associated with an unfavorable prognosis. In the current study, the authors analyze CD163 reactivity in NSCLCs and correlate with prognosis.

Methods: 342 lung adenocarcinomas and 103 squamous carcinomas identified between 2008-2013 from two different hospitals.

IHC for CD163 and numerous other antibodies were utilized.

Tumors in cell culture also analyzed.

Results: CD163 reactivity was identified in 37% adenocarcinomas and 34% squamous carcinomas.

Intensity of CD163 was lower on cancer cells than infiltrating macrophages.

CD163 stain on cancer cells was associated with significantly shorter progressions free survival and overall survival in the high reactivity group of adenocarcinomas as opposed to the low group; a similar trend was observed in squamous cell carcinoma cases, but the difference was not statistically significant.

No CD163 mRNA expression was detected in any of the cell lines suggesting that cancer cell uptake of serum CD163 and in activated macrophages accounts for the CD163 positive staining identified.

Comment: Another potential prognostic marker.

8. False-negative programmed death-ligand 1 immunostaining in ethanol-fixed endobronchial ultrasound-guided transbronchial needle aspiration specimens of non-small-cell lung cancer patients. Koomen BM, Vreuls W, de Boer M, de Ruiter EJ, Hoelters J, Vink A, Willems SM. *Histopathology*. 2021;79:480-90.

Background: The authors studied whether ethanol prefixation of clots found EBUS diminished PD-L1 immunostaining compared with formalin fixation.

Methods: FFPE from EBUS-TBNA of 54 NSCLC patients.

Paired samples were available consisting of clots directly immersed in formalin and clots prefixed in Fixcyt (50% ethanol). Serial sections were immunostained for PD-L1 using the SP263 assay and the 22C3 antibody as an LDT.

PD-L1 reactivity was determined with two cutoffs (1% and 50%).

Concordance between formalin-fixed (gold standard) and ethanol-prefixed material was assessed.

Results: 22C3 LDT:

- 30% and 36% of ethanol-prefixed specimens showed false-negative results at the 1% and 50% cutoffs
- SP263: 22% of ethanol-prefixed specimens showed false-negative results at the 1% cutoff; at 50% cutoff concordance was higher with only 12% false-negative results

Discussion: Ethanol fixation of EBUS-TBNA specimens prior to formalin fixation can result in a considerable number of false-negative PD-L1 immunostains. These results could significantly affect patient treatment and potentially outcome.

Comment: This could be particularly true in consult material, where one does not know the stains used.

9. Prognostic and predictive value of PD-L1 expression and tumour infiltrating lymphocytes (TiLs) in locally advanced NSCLC treated with simultaneous radiochemotherapy in the randomized, multicenter, phase III German Intergroup Lung Trial (GILT). Tufman A, Neumann J, Manapov F, Sellmer L, Jung A, Kauffmann-Guerrero D, Kahnert K, Mertsch P, Borgmeier A, Semrau S, Rittmeyer A, Ulm B, Ulm K, Flentje M, Fietkau R, Huber RM. *Lung Cancer*. 2021;160:17-27.

Background: Despite the fact that immune checkpoint inhibition after radiochemotherapy has become a new standard for locally advanced NSCLC with PD-L1 expression, little is known about the role of immune response markers in this setting.

The authors analyzed PD-L1 expression in tumor infiltrating lymphocytes (TiLs) from patients enrolled in a multicenter German Intergroup Lung Trial (GILT), who had been

previously randomized stage III NSCLC radiation and chemotherapy with or without consolidation chemotherapy.

Methods: Retrospective analysis using the Ventana SP263 assay. TiL score was given as (low, 0-10%; intermediate, 20-40%; or high, >50%), and pattern (excluded, inflamed, desert).

- Immune desert: little or no immune cell infiltration
- Immune excluded: immune cells aggregating at the tumor boundaries
- Inflamed tumors: showing pronounced immune infiltrate within the tumor core

Primary endpoint was impact of PD-L1 expression on progression-free survival (PFS). Secondary endpoints were overall survival and Disease Control Rate (DCR).

Results: Tumor samples available from 92 patients for a variety of reasons. TiLs could be analyzed in 66 samples among 2 of the arms of randomization in the trial (see Table 1).

Overall survival was better for those with high TiLs but the effect was not seen for PFS.

The favorable prognosis for patients with high TiL was maintained among the subgroups with the subgroup >20% having a significantly longer overall survival than the score of zero or low.

Discussion: PD-L1 expression did not correlate with PFS following radiochemotherapy. But patients with TiLs greater than 20% were found to have a longer overall survival especially for those treated with consolidation chemotherapy.

10. Trends in accuracy and comprehensiveness of pathology reports for resected NSCLC in a high mortality area of the United States. Smeltzer MP, Lee YS, Faris NR, Fehnel C, Akinbobola O, Meadows-Taylor M, Spencer D, Sales E, Okun S, Giampapa C, Anga A, Pacheco A, Ray MA, Osarogiagbon RU. *J Thorac Oncol.* 2021;16(10):1663-71.

Background: Complete and accurate pathology reports are vital for postoperative prognostication and management. This study evaluated the impact of three interventions across a diverse group of hospitals on pathology reports of resections for NSCLC.

Methods: The authors evaluated pathology reports for patients who underwent curative-intent surgical resection for NSCLC at 11 institutions within four contiguous Dartmouth Hospital Referral Regions in Arkansas, Mississippi, and Tennessee from 2004 to 2020 for completeness and accuracy, before and after the following quality improvement interventions:

- Education (feedback to heighten awareness)
- Synoptic reporting
- Lymph node specimen collection kit for surgeons.

Authors compared the six most important items in pathology reports for postoperative management including:

- Tumor size
- Histologic type
- pT-category
- pN-category
- Margin status

across the following six patient cohorts:

- Preintervention control
- Postintervention with four different combinations of interventions
- Contemporaneous nonintervention external control

Results: In the postintervention era, odds of reporting all key items were eight times higher than those in the preintervention era.

Sixfold, eightfold increase in the odds of an accurate pT- and pN-category, respectively were identified in the postintervention compared to preintervention era.

Within the intervention groups, the odds of reporting all six key items were highest in patients who received all three interventions.

Discussion: Gaps in the quality of NSCLC reporting can be identified, quantified, and corrected by “rationally designed interventions”.

The use of synoptic reporting provided the most significant boost to accurate reporting. Limitations of the study include the fact that the actual slides do not appear to have been re-reviewed. Data were retrieved from a quality database of surgical resection.

Comment: The use of synoptic reports, clearly has a benefit. However, some of the interventions included engagement of the surgeons and the use of a lymph node “kit”.

11. Spread through air spaces (STAS): can an artifact really be excluded? (Letter to the Editor). Blaauwgeers H, Thunnissen E. *Am J Surg Pathol.* 2021;45(10):1439.

Summary: There continues to be a significant amount of discussion about whether STAS is artifact or real. This Letter to the Editor takes umbrage with the recent article by Metovic, et al (*Am J Surg Pathol* 2021;45:215-222).

12. Reply to: Spread through air spaces (STAS): can an artifact really be excluded? Metovic J, Volante M, Righi L, Papotti M. *Am J Surg Pathol.* 2021;45(1):1439-40.

Summary: Additional reading in the ongoing volley about STAS.

13. Comparison of cancer control between segmentectomy and wedge resection in patients with clinical stage IA non-small cell lung cancer. Tsutani Y, Handa Y, Shimada Y, Ito H, Ikeda N, Nakayama H, Yoshimura K, Okada M. *J Thorac Cardiovasc Surg.* 2021;162:1244-52.

Summary: This study compared cancer control between segmentectomy and wedge resection in patients with clinical stage IA non-small cell lung carcinoma.

Cancer control was better in segmentectomy than in wedge resection and the authors suggest that it is the preferred procedure if sublobar resection is being performed to treat clinical stage IA carcinoma.

14. Commentary: Wedge versus segmentectomy: it is best to err on the side of caution. Hanna WC. *J Thorac Cardiovasc Surg.* 2021;162:1254
15. Commentary: Be selective when you wedge. Linden PA. *J Thorac Cardiovasc Surg.* 2021;162:1253.

Two commentaries on either side of the issue. It may be a while before these surgical debates are settled.

16. Correlating SS18-SSX immunohistochemistry (IHC) with SS18 fluorescent in situ hybridization (FISH) in synovial sarcomas: a study of 36 cases. Tay TKY, Sukma NB, Lim TH, Kuick CH, Goh JY, Chang KTE. *Virchows Archiv.* 2021;479:785-93.

Background: The standard for diagnosis of synovial sarcoma has become identification of the fusion of the SS18 gene on chromosome 18 with either SSX1, SSX2, or in rare cases SSX4 on the X chromosome. Recently, 2 novel antibodies were identified to identify the SS18-SSX fusion protein (E9X9V clone) that binds to amino acid residues surrounding the SS18-SSX fusion site, and an SSX-specific antibody (E5A2C clone) that binds the C-terminus of the SSX protein. Little is known, however, about how the SS18-SSX IHC correlates with SS18 FISH.

Methods: Archived cases of synovial sarcoma were identified; only those with prior FISH and available FFPE for SS18-SSX IHC were included. All tumors were subjected to SS18 FISH, SS18-FFX IHC (clone E9X9V) and NGS (those tumors with atypical SS18 FISH patterns).

The antibody stains the cytoplasm and typically stains tumors in a diffuse pattern with moderate intensity. Intensity appears to be affected by length of FFPE storage (embedded prior to 2016 or earlier had less intensity).

Results: 36 synovial sarcomas from 2009-2019. Ten tumors had atypical FISH patterns.

All 26 tumors with classic SS18 break-apart FISH patterns were positive for SS18-SSX IHC.

Among 10 tumors with atypical FISH patterns, 5 were positive for SS18-SSX IHC.

Among remaining 5 with atypical FISH patterns, one had a TPM3-NTRK1 fusion, and one had no fusion while the remaining 3 had insufficient tissue for sequencing.

Sensitivity of the IHC was 91% (after excluding the two cases with confirmed absence of SS18-SSX fusion).

20 histologic mimics of SS also failed to stain with IHC (100% specificity).

Discussion: The authors believe that their SS18-SSX IHC is more specific than SS18 FISH in diagnosing SS especially in those cases with an atypical FISH pattern. It correlates well with RNA sequencing and has the potential to replace SS18 FISH.

Authors suggest that a positive IHC result supports the diagnosis of SS while a tumor with an atypical FISH pattern and negative IHC should undergo further molecular testing.

The sensitivity and specificity of the SS18-SSX IHC is similar to two prior studies.

This study is unique in correlating SS18-SSX IHC with SS18 FISH patterns. A tumor with an atypical SS18 FISH pattern and negative SS18-SSX IHC should be regarded with caution as they may not represent synovial sarcomas.

Non-Neoplastic Notations

1. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-eighth adult lung transplantation report – 2021; focus on recipient characteristics. Chambers DC, Perch M, Zuckermann A, Cherikh WS, Harhay MO, Hayes Jr D, Hsich E, Khush KK, Potena L, Sadavarte A, Lindblad K, Singh TP, Stehlik J, for the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant.* 2021;40(10):1060-72.

Summary: This is the annual report on lung transplantation. It focuses on recipient characteristics. The collection period is from January 1, 1992 to June 30, 2018. Lots of the usual stats and graphs useful for talks.

2. India's second wave of coronavirus disease 2019 (COVID-19) and the lethal outbreak of black fungus infection. Agoramoorthy G. *Arch Pathol Lab Med.* 2021 Oct;145:1191-92.

Summary: Early in the pandemic the emergence of antimicrobial resistance, bacterial and fungal threats to COVID-19 were highlighted in several articles including one by the CDC. This Letter to the Editor highlights the reality in India where an outbreak of mucormycosis killed more than 2100 COVID-19 patients. Prior to the pandemic mucormycosis was quite rare in India. The letter is a call to action for increased spending on healthcare in India.

3. CT findings and patterns of e-cigarette or vaping product use-associated lung injury: a multicenter cohort of 160 cases. Kligerman SJ, Kay FU, Raptis CA, Henry TS, Sechrist JW, Walker CM, Vargas D, Filev PD, Chung MS, Digumarthy SR, Ropp AM, Mohammed TL, Pope KW, Marquis KM, Chung JH, Kanne JP. *Chest*. 2021;160(4):1492-1511.

Background: This study was done to evaluate the relative frequencies and associations with vaping behavior and imaging findings.

Methods: 160 patients with EVALI from 15 institutions.

Table 2 lists the frequency of the various patterns.

Increased frequency of vaping is associated with more extensive lung injury.

Those that had just started vaping had an increased likelihood of developing diffuse alveolar damage compared with those who have been vaping > 6 months.

Comment: Know that this is strictly a radiology study, but I believe a good reference to have handy.

Reviews and Case Reports

1. Significance of stratifin in early progression of lung adenocarcinoma and its potential therapeutic relevance. Shiba-Ishii A. *Pathol Int*. 2021;71:655-65.

Summary: Stratifin (SFN; 14-3-3-sigma) is a protein showing significantly higher expression in early invasive adenocarcinoma as opposed to AIS. Expression of SFN is controlled epigenetically by DNA demethylation and its overexpression is significantly correlated with poorer outcome. In vitro and in vivo analyses have shown that SFN facilitates early progression of adenocarcinoma by enhancing cell proliferation.

This review summarizes the genetic and epigenetic abnormalities that can occur in early-stage lung adenocarcinoma.

Article also discusses recent findings regarding the biologic significance of SFN overexpression during the course of lung adenocarcinoma progression.

Therapeutic strategies for targeting SFN are also discussed.

2. A comparison of the clinical, viral, pathologic, and immunologic features of severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and coronavirus 2019 (COVID-19) diseases. Barth RF, Buja LM, Barth AL, Carpenter DE, Parwani AV. *Arch Pathol Lab Med*. 2021;145:1194-1211.

Summary: A review article summarizing similarities and differences between SARS, MERS, and COVID-19. It doesn't seem like there is much new here but it's all in one place. Table 3 is a nice clinical summary of the three diseases. I would have liked a similar table identifying relevant pathology findings.

3. Transbronchial biopsies' histopathological findings leading to successful late steroid therapy in Covid-19 acute respiratory failure. Klein F, Soriano JC, Anfuso MB, Ruiz V, Perazzo M, Paladini H, Vigliano A, Osses J, Lowenstein P, Vigliano C, Caneva J. *Virchows Archiv.* 2021;479:827-33.

Summary: A case report of a patient with ARDS in which transbronchial biopsy demonstrated acute organizing diffuse alveolar damage. The authors do a rather extensive IHC workup on biopsies taken on day 3, 38 and 45. They showed classic progression of acute inorganizing diffuse alveolar damage. They also show a predominant CD8 positive lymphocytes in both early and late phases, different from what has been previously reported. In the late phases there appeared to be more inflammatory cells than early.

Comment: Interesting case report given with sequential findings.

4. Liquid biopsy for advanced NSCLC: a consensus statement from the International Association for the Study of Lung Cancer. Rolfo C, Mack P, Scagliotti GV, Aggarwal C, Arcila ME, Barlesi F, Bivona T, Diehn M, Dive C, Dziadziuszko R, Leighl N, Malapelle U, Mok T, Peled N, Raez L, Sequist L, Sholl L, Swanton C, Abbosh C, Tan D, Wakelee H, Wistuba I, Bunn R, Freeman-Daily J, Wynes M, Belani C, Mitsudomi T, Gandara D. *J Thorac Oncol.* 2021;16(10):1647-62.

Summary: A nice overview of the state of the art.

5. Aspergillus infections. Thompson III GR, Young JAH. *N Engl J Med.* 2021;385:1496-509.

Summary: Great diagrams potentially useful for teaching.

6. Squamous differentiation in surgically resected malignant pleural mesothelioma with neoadjuvant chemotherapy. Yotsukura M, Motoi N, Yoshida A, Yoshida Y, Nakagawa K, Watanabe S. *Pathol Int.* 2021;71:692-6.

Summary: Another case report of squamous differentiation in mesothelioma. The tubulopapillary component was positive for CK5/6, WT-1 and calretinin, while negative for TTF-1, claudin-4 and p40.

Squamous cells were positive for cytokeratin 5/6 and p40 but negative for WT-1, calretinin, and TTF-1. Loss of BAP1 was observed in both tubulopapillary and squamous tumor cells.

7. Clinical-pathologic challenges in the classification of pulmonary neuroendocrine neoplasms and targets on the horizon for future clinical practice. Derks JL, Rijnsburger N, Hermans BCM, Moonen L, Hillen LM, von der Thusen JH, den Bakker MA, van Suylen RJ, Speel EJM, Dingemans AMC. *J Thorac Oncol.* 2021;16(10):1632-46.

Summary: A summary of current state pulmonary neuroendocrine tumors which seeks to answer the following five questions:

- What is the preferred outcome parameter for curatively resected low-grade neuroendocrine neoplasms (overall survival vs recurrence-free survival)?
- Does the WHO classification combined with a Ki-67 proliferative index and molecular markers, such as OTP and CD44, offer improved prognostication for low-grade NET?
- What is the value of a typical versus atypical carcinoid diagnosis on a biopsy specimen in local and metastatic disease?
- What is the relevance of separating large cell neuroendocrine carcinoma from small cell carcinoma and the value of molecular markers such as RB1 gene and pRb protein, or transcription factors NEUROD1, ASCL1, POU2F3, or YAP1 to predict systemic treatment outcome?
- Are additional diagnostic criteria required to accurately separate LCNEC from NSCLC in biopsy specimens?