

Pulmonary Journal Club March 2021 (Articles from February 2021)

Presented by

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Articles for Discussion

Chapel DB. Malignant peritoneal mesothelioma: prognostic significance of clinical and pathologic parameters and validation of a nuclear-grading system in a multi-institutional series of 225 cases. Modern pathology. 2021; 34(2):380-395. Discussed by L. Schrader

Purpose: To define the prognostic significance of clinical, morphologic, and immunophenotypic features of malignant peritoneal mesothelioma.

Methods: Retrospective, multi-institutional study of 225 malignant peritoneal mesotheliomas. Parameters were defined for clinical features, surgical course, imaging, morphology and IHC. Under the morphologic parameters a composite score was generated based on nuclear grading and mit/10 HPF (MSKCC composite system). The parameters defined in IHC included BAP1. Univariate and multivariate analysis were performed between the parameters defined within the various categories and overall survival.

Results:

On univariate analysis longer overall survival was significantly associated with:	P value
Diagnosis after 2000	P =0.0001
Age <60	P =0.0001
ECOG performance status	P =0.01
Absence of radiographic lymph node metastasis	P =0.04
Cytoreduction surgery	P =0.0001
Hyperthermic intraperitoneal chemotherapy	P =0.0001
Peritoneal carcinomatosis index <27	P =0.01
Absence of necrosis	P =0.007
Epithelioid histology	P =0.0001

Overall survival among epithelioid malignant mesotheliomas was significantly associated with:	P value
Female sex	P =0.03
Tubulopapillary architecture	P =0.005
Low pleomorphism	P <0.0001
Low mitotic index	P =0.0007
Low composite nuclear grade	P <0.0001

- BAP1 loss is not associated /w improved prognosis in peritoneal mesothelioma.
- Necrosis is not an independent prognostic indicator in peritoneum epithelioid mesothelioma

Discussion: This study further defines the clinical and pathologic prognostic indicators in peritoneal mesothelioma. The MSKCC composite nuclear grading system could be applicable to both pleural and peritoneal epithelioid mesotheliomas.

Metovic J. Gross Specimen Handling Procedures Do Not Impact the Occurrence of Spread Through Air Spaces (STAS) in Lung Cancer. Am J Surg Pathol. 2021; 45(2):215-222.

Background:

- The presence of STAS is generally considered as one of the manifestations of lung cancer aggressiveness.
- The real nature of the STAS phenomenon is widely debated in the literature and the mechanism of STAS is yet to be understood.
- Loose tumor fragments can be misplaced during tissue manipulation by the surgeon and/or in the pre-analytical phase of gross specimen handling, versus a biological event linked to intrinsic tumor invasion ability.

Aim:

- To investigate the potential impact of gross sampling procedures in the pathology laboratory in causing STAS in a series of resected lung cancers.

Methods:

- Prospective series of 51 surgically resected fresh lung specimens, harboring an easily palpable tumor mass, and with sufficient lung parenchyma surrounding the neoplasia (not those with extensive visceral pleura infiltration/retraction), excluding neoadjuvant chemotherapy.
- Using a new and clean blade, the first cut was performed perpendicular to the pleura through the peripheral parenchyma, the tumor mass, and the deep lung parenchyma. Replacing the blade with a new one, a second cut was done along the same direction. Resulting in (i) fresh upper sample, and (ii) fresh lower sample. The residual specimen was then fixed and 2 additional sections parallel to the previous fresh-cut surface were obtained using a clean blade each time - (iii) fixed upper sample and (iv) fixed lower sample.
- Clinicopathologic data, histology of the tumor, STAS characteristics, peritumoral lung tissue alterations were recorded.

Results:

- 33/51 (64.7%) cases are STAS positive.
- In STAS-positive cases, its presence was found in 20/33 (60.6%) upper fresh samples and in 19/31 upper fixed samples (61.3%). Regarding lower portions, it was observed in 21/33 lower fresh samples (63.6%) and 21/32 lower fixed samples (65.6%).
- The STAS predominant pattern was cluster formation (29 cases, 87.9%)

- STAS was observed in the nearby alveoli in 16 cases (48.5%) (2nd-5th alveolar space) and also in distant air spaces (beyond 5th alveolar space) in the other 17 cases (51.5%).
- No significant difference in peritumoral lung tissue alterations was noted between STAS-positive and STAS-negative cases
- The median value of tumor clusters per case was 9 (range: 2 to 92) and the mean value was 21.5±23.3 clusters

Discussion:

- In STAS-positive cases, its presence was found in 20/33 (60.6%) upper fresh samples and in 21/33 lower fresh samples (63.6%). Regarding fixed samples, STAS was found in 19/31 upper fixed samples (61.3%) and in 21/32 lower fixed samples (65.6%).
- No significant association was observed between STAS status and clinicopathologic characteristics
- Correlation with clinicopathologic parameters did not show any significant differences between the cases pertinent to the high and low STAS groups

Take Home Points:

- STAS most probably is not a pathologist-related artifactual event due to knife transportation of tumor cells during gross specimen handling.

Invernizzi R. The Respiratory Microbiome in Chronic Hypersensitivity Pneumonitis Is Distinct from That of Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med. 2021; 203(3):339-347.

Background:

- Studies of the respiratory microbiome in idiopathic pulmonary fibrosis (IPF) have reported associations between changes in the bacterial burden, microbial communities, disease progression, and mortality.
- Chronic hypersensitivity pneumonitis (CHP) is a pathogenically and prognostically distinct disorder compared with IPF but is still characterized phenotypically by irreversible fibrotic destruction of the lung parenchyma.
- Despite the phenotypic overlap, no study has examined whether the respiratory microbiota differs between individuals with these conditions.

Aim:

- To characterize and compare the airway microbiome in subjects with CHP, subjects with idiopathic pulmonary fibrosis (IPF), and control subjects.

Methods:

- Prospectively recruited individuals with a CHP diagnosis ($n = 110$), individuals with an IPF diagnosis ($n = 45$), and control subjects ($n = 28$).
- Subjects underwent BAL and bacterial DNA was isolated, quantified by quantitative PCR and the 16S ribosomal RNA gene was sequenced to characterize the bacterial communities in the lower airways.

Results:

- At the phylum level, the prevailing microbiota of both subjects with IPF and subjects with CHP included *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*.
- In CHP there are increased proportions of *Proteobacteria* and lower proportions of *Firmicutes* compared with subjects with IPF.
- At the genus level, the *Staphylococcus* burden was increased in CHP, and *Actinomyces* and *Veillonella* burdens were increased in IPF.
- The lower airway bacterial burden in subjects with CHP was higher than that in control subjects but lower than that of those with IPF.
- In contrast to IPF, there was no association between bacterial burden and survival in CHP.

Discussion:

- Subjects with IPF were predominantly male (82%) and moderately severe restrictive lung disease. There were more female subjects in the CHP cohort, and although their mean age and DL_{CO} were matched, they had a more preserved FVC compared with the IPF cohort.
- 16S rRNA gene sequencing provides a snapshot of the microbial community at a particular moment in time, and it is therefore challenging to draw conclusions regarding whether the altered microbial community is a cause or a consequence of the disease
- Healthy control subjects totally healthy volunteer

Take Home Points:

- The microbial profile of the lower airways in subjects with CHP is distinct from that of IPF
- The bacterial burden in individuals with CHP fails to predict survival.

Chen C-L. An annotation-free whole-slide training approach to pathological classification of lung cancer types using deep learning. Nat Commun. 2021; 12(1):1193

Background:

- Deep neural networks (DNNs), especially convolutional neural networks (CNNs), have become the dominant method for image recognition. However, it is hindered by the extremely high spatial resolution of whole-slide images (WSIs).
- Most studies have employed patch-based methods, which often require detailed annotation and laborious free-hand contouring of image patches.

- To reduce the annotation burden, multiple-instance learning (MIL) follows the same two-stage workflow as the traditional method. Using pre-trained conventional neural network classifier and using only the slide-level ground truth as weak supervision. If patches with the highest scores (k patches that are most likely to be cancerous) on the slide are identified as carcinoma, the slide should be classified as cancer. However, the performance still cannot achieve the average performance of strong supervision methods.

Aim:

- To train standard CNNs with extremely large image inputs without modification in either training pipelines or model architectures – no dividing into patches.

Methods:

- Developed a whole-slide training method that incorporates the unified memory (UM) mechanism: using system memory – however very slow.
- Several GPU memory optimization techniques: group execution (concurrent execution of operations only exists within a group scope to decrease moving back and forth between memories) and group prefetch (preload data required by the upcoming operations in advance to prevent memory stalls).

Results:

- Proposed method has superior performance, achieving area under the receiver operating characteristic curve (AUC) scores of 0.9594 and 0.9414 for adenocarcinoma and squamous cell carcinoma classification, respectively.
- Critical regions of the model highlighted by the class activation map (CAM) technique reveal a high correspondence to cancerous regions identified by pathologists.

Take Home Points:

- If combined with strongly supervised methods, it would be able to leverage a large amount of weakly labeled data to achieve a crude understanding.
- Methods like this will greatly speed up the pathology AI progress.

Lin Y-Y. Programmed death-ligand 1 (PD-L1)/thyroid transcription factor-1 double immunohistochemical staining facilitates scoring of tumor PD-L1 expression in cytopathology specimens from lung adenocarcinoma patients. Cancer Cytopathol. 2021; 129(2):148-155.

Background:

- PD-L1 evaluation relies on pathologists' visual estimation of tumor PD-L1 staining, which can be variable in certain conditions: mesothelial cells, macrophages, and etc..

Aim:

- Highlighting tumor cells via double immunostaining with PD-L1 and TTF-1 may improve estimation accuracy.

Methods:

- Performed PD-L1 single staining and PD-L1/TTF-1 double staining in 42 pairs of cytopathology and histopathology specimens from lung adenocarcinoma patients.
- An experienced pathologist visually estimated PD-L1 expression in each case and placed tumor PD-L1 expression into 1 of 3 categories: <1%, 1%-49%, or \geq 50%.
- A medical technologist also performed estimations of the same cases based on a count of 200 tumor cells, and the results were compared.

Results:

- The concordance of PD-L1 expression categorization between the pathologist's visual estimation and the medical technologist's counting was increased by double staining in cytopathology specimens
- Double staining reduced possible error in the pathologist's visual estimation of PD-L1 expression from 9.5% to 4.8%.
- The benefit was not observed in histopathology specimens.

Take Home Points:

- PD-L1/TTF-1 double immunohistochemistry technique can be applied successfully to cytopathology specimens
- I have been personally planning to do a similar but different study. Please contact me if you are interested in collaborating!

Terra SBSP. Utility of Immunohistochemistry for MUC4 and GATA3 to Aid in the Distinction of Pleural Sarcomatoid Mesothelioma From Pulmonary Sarcomatoid Carcinoma. Arch Pathol Lab Med. 2021; 145(2):208-213.

Background:

- Distinguishing pulmonary sarcomatoid carcinoma from pleural sarcomatoid mesothelioma is challenging because of overlapping histology, immunophenotype, and clinical features.

Aim:

- To explore the utility of MUC4 and GATA3 in distinguishing pulmonary sarcomatoid carcinoma from sarcomatoid mesothelioma.

Methods:

- Well-characterized cases of sarcomatoid carcinoma (n = 32) and sarcomatoid mesothelioma (n = 64) were included.

- Diagnoses were confirmed by thoracic pathologists with incorporation of immunophenotype, clinical, and radiographic features. Whole-tissue sections were stained for GATA3 and MUC4.

Results:

- Patients with sarcomatoid carcinoma and sarcomatoid mesothelioma had similar mean age and male predominance.
- GATA3 was positive in 63 of 64 sarcomatoid mesotheliomas (98%; 42 diffuse, 16 patchy, 5 focal), and 15 of 32 sarcomatoid carcinomas (47%; 3 diffuse, 8 patchy, 4 focal).
- MUC4 was positive in 2 of 64 sarcomatoid mesotheliomas (3%; 1 patchy, 1 focal), and in 12 of 32 sarcomatoid carcinomas (38%; 5 diffuse, 6 patchy, 1 focal).

Take Home Points:

- Diffuse GATA3 expression favors sarcomatoid mesothelioma over sarcomatoid carcinoma, which rarely shows diffuse expression.
- Focal and patchy GATA3 expression is observed in both tumor types, and therefore is not helpful in this distinction.
- Sensitivity of MUC4 for sarcomatoid carcinoma was low but it was quite specific.

Okudela K. A method to obtain reproducible Ki-67 indices in lung adenocarcinoma. *Histopathology*. 2021; 78(3):414-423.

Background:

- Proliferative activity, evaluated from the Ki-67 index, is a strong prognostic factor in lung adenocarcinoma (LADC).

Aim:

- To optimize a procedure to measure the Ki-67 index and establish the best cut-off value.

Methods:

- 342 stage I LADCs for the immunohistochemical expression of Ki-67 using different antibodies, MIB1 and SP6. Superior specificity of SP6; therefore, SP6 was used.
- Slides were scanned with a virtual slide system. Using software, tumor cells were counted in a whole tumor. Thereafter, the tumor was evenly subdivided into 0.25-mm² tiles. The frequency of positive cells was counted in each tile of an invasive area or the whole tumor.
- Calculated the number of tumor cells required to produce a 95% confidence interval (CI) <0.05. Additionally, calculated coverage probabilities (CP) using two different methods, counting any number or 200 cells per tile.

Results:

- Need to count 2000 cells from 10 random tiles (200 cells each) in invasive areas to achieve CP > 70% and CI of <0.05 in more than 90% of cases.
- An optimal cut-off value of 0.12 with an alternative of 0.15, based on disease recurrence.

Take Home Points:

- A procedure and the cut-off values may be used in the routine pathological diagnosis of LADC.

Herbst RS. Phase 1 Expansion Cohort of Ramucirumab Plus Pembrolizumab in Advanced Treatment-Naive NSCLC. J Thorac Oncol. 2021; 16(2):289-298.

Background:

- Responses to checkpoint inhibitor therapy have improved with the addition of vascular endothelial growth factor (VEGF) inhibitors or VEGF receptor-2 (VEGFR2) inhibitors, possibly by increasing access of T cells to tumors and blockade of immunosuppressive cytokines and regulatory T cells in the tumor microenvironment.

Aim:

- To provide data of first-line ramucirumab plus pembrolizumab treatment of programmed death-ligand 1 (PD-L1)-positive NSCLC

Methods:

- Multicenter, open-label phase 1a/b trial, patients received ramucirumab and pembrolizumab every 21 days for up to 35 cycles.
- PD-L1 positivity was defined as tumor proportion score (TPS) greater than or equal to 1%.
- Exploratory NanoString biomarker analyses included three T-cell signatures (T-cell-inflamed, Gajewski, and effector T cells) and CD274 gene expression.

Results:

- 26 patients.
- Treatment-related adverse events of any grade occurred in 22 patients (84.6%). Treatment-related adverse events of grade greater than or equal to 3 were reported in 11 patients (42.3%); the most frequent was hypertension (n = 4, 15.4%).
- Objective response rate was 42.3% in the treated population and 56.3% and 22.2% for patients with high (TPS ≥ 50%) and lower levels (TPS 1%–49%) of PD-L1 expression, respectively.
- Median progression-free survival (PFS) in the treated population was 9.3 months, and 12-month and 18-month PFS rates were 45% each.
- Median PFS was not reached in patients with PD-L1 TPS greater than or equal to 50% and was 4.2 months in patients with PD-L1 TPS 1% to 49%.

- Median overall survival was not reached in the treated population, and 12-month and 18-month overall survival rates were 73% and 64%, respectively.
- Biomarker data suggested a positive association among clinical response, three T-cell signatures, CD274 gene expression, and PD-L1 immunohistochemistry.

Take Home Points:

- First-line therapy with ramucirumab plus pembrolizumab has a manageable safety profile in patients with NSCLC, and the efficacy signal seems to be strongest in tumors with high PD-L1 expression.

Jiang T. Mutational Landscape and Evolutionary Pattern of Liver and Brain Metastasis in Lung Adenocarcinoma. *J Thorac Oncol.* 2021; 16(2):237-249.

Background:

- The genetic divergence and phylogenetic relationships among diverse metastatic lesions from cancer remains limited.

Aim:

- A comprehensive genomic analysis of paired primary tumors and their metastatic lesions may provide new insights into the biology of metastatic processes and therefore guide the development of novel strategies for intervention.

Methods:

- Whole-exome sequencing in 84 tissue and blood samples from 26 patients with lung adenocarcinoma having liver metastases (LiM) or brain metastases (BrM) before any systemic therapy.
- Mutational landscape and evolutionary patterns were compared between paired primary lesions (primary lesion of LiM or BrM) and metastases (metastatic site of LiM or BrM).

Results:

- Common driver mutations, including *TP53* and *EGFR*, were highly consistent between paired primary and metastatic tumors.
- Tumor mutational burden was comparable among groups, the LiM group had significantly higher mutational and copy number variational similarity than the BrM group between paired primary lesions and metastases ($p = 0.019$ and $p = 0.035$, respectively).
- Phylogenetic analysis further revealed that LiM-competent disseminations had a higher level of genetic similarity to their paired primary lesions and were genetically diverged from their primary tumors at a relatively later stage than those of BrM.

Take Home Points:

- Mutational landscape and evolutionary pattern was distinctly different between the LiM and BrM of lung adenocarcinoma: LiM favorably followed the linear progression model, whereas BrM was more consistent with the parallel progression model.

Yanagawa N. Programmed death ligand 1 protein expression is positively correlated with the solid predominant subtype, high MIB-1 labeling index, and p53 expression and negatively correlated with epidermal growth factor receptor mutations in lung adenocarcinoma. Hum Pathol. 2021; 108:12-21.

Background:

- Therapeutic effect of immunotherapy using immune checkpoint antibodies is closely related to PD-L1 expression in cancer cells, and PD-L1 protein expression has been suggested to be a predictive biomarker of the response to immunotherapy.

Aim:

- To identify the clinicopathological and molecular characteristics associated with PD-L1 expression

Methods:

- patients with surgically resected nonsmall cell lung carcinoma

Results:

- Among 633 patients with adenocarcinoma, 523 (82.6%) had no PD-L1 expression, 78 (12.3%) low expression, and 32 (5.1%) high expression.
- PD-L1 expression was more common in men ($p < 0.001$), in smokers ($p = 0.002$), and in patients with a more advanced stage ($p = 0.002$), the solid predominant subtype ($p < 0.001$), no epidermal growth factor receptor (*EGFR*) mutations ($p < 0.001$), a high MIB-1 labeling index ($p < 0.001$), and positive p53 immunohistochemical expression ($p < 0.001$).
- In the 193 patients with squamous cell carcinoma, 92 (47.7%) had no PD-L1 expression, 57 (29.5%) low expression, and 44 (22.8%) high expression.
- There were no significant correlations between PD-L1 expression and the evaluated clinicopathological or molecular characteristics of these patients.

Take Home Points:

- Their PD-L1 positive rate is significantly lower than others.
- Associations of PD-L1 with various clinicopathological or molecular characteristics in adenocarcinoma but not squamous cell carcinoma.

Liu L. Clinicopathological features and prognostic analysis of 247 small cell lung cancer with limited-stage after surgery. Hum Pathol. 2021; 108:84-92.

Background:

- There have been only modest improvements in survival for patients with small cell lung cancer (SCLC)
- However, until the release of the 2002 National Comprehensive Cancer Network guidelines, radical surgery was not the recommended treatment option for limited-stage SCLC.

Aim:

- To analyze the clinical and pathological characteristics of patients with small cell lung cancer (SCLC) after curative surgery and to explore prognostic factors for disease-free survival (DFS) and overall survival (OS).

Methods:

- 247 patients were collected, and clinicopathological features were retrieved, including gender, age, smoking history, tumor location, and distant metastasis.
- Histopathological features were also reviewed by three pathologists, including primary tumor (T), lymph node metastasis (N), pleural invasion, bronchial invasion, nerve invasion, spread through air spaces (STAS), tumor thrombosis, major cell shape (round Vs. spindle), tumor necrosis, stromal fibrosis, and tumor-infiltrating lymphocytes (TILs).
- Immunohistochemical staining of neuroendocrine markers (CD56, synaptophysin, chromogranin A) was also reviewed.
- All patients were followed up for recurrence, distant metastasis, and survival.

Results:

- The median DFS was 98 months, and the 1-year, 3-year, and 5-year DFS rates were 70.9%, 54.4%, and 52.2%, respectively. The median OS was not reached, and the 1-year, 3-year, and 5-year survival rates were 94.2%, 72.3%, and 65.4%, respectively.
- Age more than 65 years, smoking, advanced stage (T and N), distant metastasis, nerve invasion, major cell shape as spindle and TILs >30% were negatively correlated with survival.
- Neuroendocrine immunostaining markers showed no correlation with survival.

Take Home Points:

- Spindle cell type and TILs >30% are revealed as independent negative prognostic factors.

Chae M. Prognostic significance of tumor spread through air spaces in patients with stage IA part-solid lung adenocarcinoma after sublobar resection. Lung Cancer. 2021; 152:21-26.

Background:

- Several studies reported that the presence of STAS was a risk factor for cancer recurrence and lymph node metastasis in lung adenocarcinoma treated with anatomic pulmonary resection.
- Few reports have investigated the clinical impacts of STAS for patients with early stage part-solid lung adenocarcinoma after sublobar resection.

Aim:

- To assess the clinicopathologic implications of STAS in patients with stage IA part-solid lung adenocarcinoma after sublobar resection.

Methods:

- Medical records of patients with stage IA part-solid adenocarcinoma who underwent curative pulmonary resection between February 2009 and December 2016 were retrospectively reviewed.
- The clinicopathological features of STAS and its influence on postoperative recurrence and survival were investigated.

Results:

- 115 patients with stage IA part-solid adenocarcinoma who underwent wedge resection, 20 (17.4 %) had STAS.
- The multivariable analysis showed presence of STAS [HR (hazard ratio), 9.447; $p = 0.002$] and a larger invasive component size (HR, 1.097; $p = 0.034$) were independent risk factors for recurrence.
- The 5-year freedom from recurrence rates were 62.4 % and 97.9 % in cases with and without STAS, respectively ($p < 0.001$), and the 5-year disease-free survival rates were 58.5 % and 97.9 % in cases with and without STAS, respectively ($p < 0.001$).
- The presence of STAS was associated with old age ($p = 0.030$), male gender ($p = 0.023$), acinar predominant histologic pattern ($p = 0.004$), presence of micropapillary pattern ($p < 0.001$), lymphovascular invasion ($p < 0.001$) and larger invasive component ($p < 0.001$).

Take Home Points:

- STAS could be an important prognostic factor in patients with stage IA part-solid lung adenocarcinoma after sublobar resection.

Patnaik SK. Lower airway bacterial microbiome may influence recurrence after resection of early-stage non–small cell lung cancer. J Thorac Cardiovasc Surg. 2021; 161(2):419-429.e16.

Background:

- Human beings exist in an intimate symbiotic relationship with bacteria. It is also evident that this relationship is altered in and perhaps causative to human pathologic processes.
- The lower airway bacterial microbiome influences carcinogenesis and response to immunotherapy in non–small cell lung cancer (NSCLC).

Aim:

- To investigate the association of this microbiome with recurrence in early NSCLC.

Methods:

- Microbiomes of presurgery bronchoalveolar lavage (BAL) and saliva, and resected stage I NSCLC tumor and adjacent lung tissues of 48 patients were examined by *16S* gene sequencing.
- Tumor gene expression was measured by RNA sequencing.

Results:

- Spatial relationships of the different biospecimen types was reflected in their microbiomes, with microbiomes of BAL intermediate to those of saliva and lung tissue.
- BAL and saliva microbiomes were less dissimilar in patients with high α -amylase levels in BAL, indicating oral aspiration as a source of lower airway microbiota.
- BAL microbiomes of patients with recurrence within 32 months of surgery differed from those without recurrence during ≥ 32 months of follow-up ($n = 18$ each), despite no difference for age, sex, smoking history, and tumor histology and grade.
- The recurrence-associated BAL microbiome signature was present in 16 of the 18 recurrence cases but in only two of the others.
- Signature presence was associated with shorter recurrence-free survival (log-rank test $P < .001$; hazard ratio = 14.5), and greater expression in tumors of genes for cell proliferation and epithelial mesenchymal transition.
- Immune cellular composition of the tumor microenvironment was not different between patients with and without the signature.

Take Home Points:

- Presurgery composition of lower airway microbiome may be associated with recurrence of early NSCLC.

Mehta A. Non-amplification genetic alterations of *HER2* gene in non-small cell lung carcinoma. J Clin Pathol. 2021; 74(2):106-110.

Background:

- *HER2* gene amplification has been observed in lung adenocarcinoma (LUAD). However, these have so far not been found actionable.⁵
- Contrarily, *HER2* missense and in-frame insertions in LUAD are being recognized as actionable.

Aim:

- To investigate the incidence and spectrum of human epidermal growth factor receptor 2 (*HER2*) mutations, associated clinicopathological characteristics and the co-occurrence of *HER2* gene amplification in the *HER2* gene mutated cases in non-small cell lung cancer (NSCLC).

Methods:

- All patients with advanced lung adenocarcinoma (LUAD) who underwent broad genomic profiling by next generation sequencing (NGS) from 2015 to 2019 were included in the study.
- *HER2* gene amplification was checked in all the *HER2* gene mutated cases.
- Tumor tissues of all the mutated cases were examined by fluorescent in situ hybridization (FISH).

Results:

- Fifty-four (37.2%) out of the 145 cases harbored tier 1 driver mutations comprising *EGFR* in 22.1%, *ALK* rearrangements in 7.6% cases, *ROS1* rearrangements and *BRAF*^{V600E} in 3.5% cases each, and *NTRK* fusion in 0.7% cases.
- Nine (6.2%) cases exhibited a significant genetic alteration in *HER2* gene (tiers 2 and 3) on NGS.
- The most common alteration was exon 20 insertion of amino acid sequence AYVM in five cases (p.E770_A771insAYVM) followed by insertion of YVMA (p.A771_Y772insYVMA) in one case, insGSP (p.V777_G778insGSP) in one case and two missense mutations: p.G776C and p.QA795C (novel variant).
- The median copy number of the *HER2* gene was 3.21 while on FISH, the median *HER2/CEP17* ratio was 2.0.

Take Home Points:

- There is a relatively higher occurrence of *HER2* exon 20 mutations as primary oncogenic driver in NSCLC especially LUAD.
- p.E770_A771insAYVM as the strikingly dominant insertion mutation against the most often globally reported p.A771_Y772insYVMA.

Hirose T. Extensive functional evaluation of exon 20 insertion mutations of *EGFR*. *Lung Cancer*. 2021; 152:135-142.

Background:

- Exon 20 insertion mutations of epidermal growth factor receptor (EGFR) have been identified as oncogenic mutations in general.
- The functional relevance of each remains largely uninvestigated.

Aim:

- To comprehensively assess the functional significance of insertion mutations of *EGFR* exon 20.

Methods:

- The transforming potential and drug sensitivities of 25 *EGFR* recurrent mutants, including twenty-one exon 20 insertions, were evaluated using the mixed-all-nominated-in-one method.

Results:

- The sensitivity of *EGFR* exon 20 insertions to EGFR tyrosine kinase inhibitors (TKIs) was generally lower than that of the L858R mutation or exon 19 deletions.
- All of the exon 20 insertions were resistant to gefitinib and afatinib, whereas several mutants were sensitive to osimertinib.
- *EGFR* exon 20 insertions exhibited relatively good responses to poziotinib and mobocertinib.

Take Home Points:

- *EGFR* exon 20 insertions were shown to have different degrees of sensitivity to EGFR TKIs.

Juge P-A. Methotrexate and rheumatoid arthritis associated interstitial lung disease. *Eur Respir J*. 2021; 57(2):2000337.

Background:

- Methotrexate (MTX) is a key anchor drug for rheumatoid arthritis (RA) management.
- Fibrotic interstitial lung disease (ILD) is a common complication of RA.
- Whether MTX exposure increases the risk of ILD in patients with RA is disputed.

Aim:

- To evaluate the association of prior MTX use with development of RA-ILD.

Methods:

- Through a case-control study design with discovery and international replication samples, the authors examined the association of MTX exposure with ILD in 410 patients with chronic fibrotic ILD associated with RA (RA-ILD) and 673 patients with RA without ILD.
- Estimates were pooled over the different samples using meta-analysis techniques.

Results:

- Analysis of the discovery sample revealed an inverse relationship between MTX exposure and RA-ILD (adjusted OR 0.46, 95% CI 0.24-0.90; p=0.022), which was confirmed in the replication samples (pooled adjusted OR 0.39, 95% CI 0.19-0.79; p=0.009).
- The combined estimate using both the derivation and validation samples revealed an adjusted OR of 0.43 (95% CI 0.26-0.69; p=0.0006).
- MTX ever-users were less frequent among patients with RA-ILD compared to those without ILD, irrespective of chest high-resolution computed tomography pattern.
- In patients with RA-ILD, ILD detection was significantly delayed in MTX ever-users compared to never-users (11.4±10.4 years and 4.0±7.4 years, respectively; p<0.001).

Take Home Points:

- MTX use is not associated with an increased risk of RA-ILD in patients with RA, and that ILD was detected later in MTX-treated patients.

Hodge G. Bronchiolitis obliterans syndrome is associated with increased senescent lymphocytes in the small airways. J Heart Lung Transplant. 2021; 40(2):108-119.

Background:

- Immunosuppression therapy is ineffective at preventing bronchiolitis obliterans syndrome (BOS), primarily a disease of the small airways (SAs).
- Previous reports show increased senescent CD28null T and natural killer T (NKT)-like cells in the peripheral blood of patients with BOS and increased cytotoxic, proinflammatory lymphocytes in the SAs.

Aim:

- To test the hypothesis that the cytotoxic, proinflammatory lymphocytes in the SAs would be steroid-resistant senescent CD28null lymphocytes.

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Methods:

- Intracellular cytotoxic mediator granzyme B, interferon (IFN)- γ and tumor necrosis factor (TNF)- α proinflammatory cytokines, and CD28 were measured in the blood, bronchoalveolar lavage, large airway, and SA brushing T and NKT-like cells from 10 patients with BOS, 11 stable lung transplant recipients, and 10 healthy age-matched controls.

- SA brushings were cultured in the presence of ± 1 $\mu\text{mol/liter}$ prednisolone, ± 5 mg/liter theophylline, and ± 2.5 ng/ml cyclosporine A, and IFN- γ and TNF- α proinflammatory cytokines were assessed using flow cytometry.

Results:

- Increased SA CD28null T and NKT-like cells were identified in patients with BOS compared with that in the controls and stable transplant recipients.
- Loss of CD28 was associated with increased T and NKT-like cells expressing granzyme B, IFN- γ , and TNF- α . Loss of CD28 expression by CD8+ T cells was significantly associated with forced expiratory volume in 1 sec ($R = 0.655$, $p = 0.006$) and with time after transplantation ($R = -0.552$, $p = 0.041$).
- Treatment with prednisolone + theophylline + cyclosporin A inhibited IFN- γ and TNF- α production by SA CD28null CD8+ T and NKT-like cells additively.

Take Home Points:

- BOS is associated with the loss of CD28 in SA cytotoxic, proinflammatory senescent T and NKT-like lymphocytes.

Maher TM. Phase 2 trial to assess lebrikizumab in patients with idiopathic pulmonary fibrosis. Eur Respir J. 2021; 57(2):1902442.

Background:

- Pirfenidone is one of two approved antifibrotic therapies for IPF.
- Interleukin (IL)-13 is a potent activator of fibroblasts, promoting extracellular matrix synthesis with potential pathogenic roles in fibrosis.
- Lebrikizumab is a humanized monoclonal antibody that specifically binds soluble IL-13 to neutralize its activity and inhibit subsequent downstream signaling.

Aim:

- A phase 2, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of lebrikizumab, an interleukin (IL)-13 monoclonal antibody, alone or with background pirfenidone therapy, in patients with idiopathic pulmonary fibrosis (IPF).

Methods:

- Patients with IPF aged ≥ 40 years with forced vital capacity (FVC) of 40%–100% predicted and diffusing capacity for carbon monoxide of 25%–90% predicted and who were treatment-naïve (cohort A) or receiving pirfenidone (2403 mg/day ; cohort B) were randomized 1:1 to receive lebrikizumab 250 mg or placebo subcutaneously every 4 weeks.
- The primary endpoint was annualized rate of FVC % predicted decline over 52 weeks.

Results:

- The primary endpoint (annualized rate of FVC % predicted decline) was not met in cohort A (lebrikizumab *versus* placebo, -5.2% *versus* -6.2% ; $p=0.456$) or cohort B (lebrikizumab *versus* placebo, -5.5% *versus* -6.0% ; $p=0.557$).
- In cohort B, a non-statistically significant imbalance in mortality favoring combination therapy was observed (hazard ratio 0.42 (95% CI 0.17–1.04)).
- Pharmacodynamic biomarkers indicated lebrikizumab activity.
- The safety profile was consistent with that in previous studies of lebrikizumab and pirfenidone as monotherapies.

Take Home Points:

- Lebrikizumab alone or with pirfenidone was not associated with reduced FVC % predicted decline over 52 weeks despite evidence of pharmacodynamic activity.
- Lebrikizumab was well tolerated with a favorable safety profile.
- Blocking IL-13 may not be sufficient to achieve a lung function benefit in patients with IPF.

Espindola MS. Differential Responses to Targeting Matrix Metalloproteinase 9 in Idiopathic Pulmonary Fibrosis. *Am J Respir Crit Care Med.* 2021; 203(4):458-470.

Background:

- Aberrant lung remodeling in idiopathic pulmonary fibrosis (IPF) is characterized by elevated MMP9 (matrix metalloproteinase 9) expression.
- The precise role of this matrix metalloproteinase in this disease has yet to be fully elucidated.

Aim:

- To evaluate antifibrotic effects of MMP9 inhibition on IPF.

Methods:

- Quantitative genomic, proteomic, and functional analyses both *in vitro* and *in vivo* were used to determine MMP9 expression in IPF cells and the effects of MMP9 inhibition on profibrotic mechanisms.

Results:

- MMP9 expression was increased in airway basal cell (ABC)-like cells from IPF lungs compared with ABC cells from normal lungs.
- The inhibition of MMP9 activity with an anti-MMP9 antibody, andecaliximab, blocked TGF- β 1 (transforming growth factor β 1)-induced Smad2 phosphorylation.
- However, in a subset of cells from patients with IPF, TGF- β 1 activation in their ABC-like cells was unaffected or enhanced by MMP9 blockade (i.e., nonresponders).

- Further analysis of nonresponder ABC-like cells treated with andecaliximab revealed an association with type 1 IFN expression, and the addition of IFN α to these cells modulated both MMP9 expression and TGF- β 1 activation.
- The inhibition of MMP9 ameliorated pulmonary fibrosis induced by responder lung cells but not a nonresponder in a humanized immunodeficient mouse model of IPF.

Take Home Points:

- MMP9 regulates the activation of ABC-like cells in IPF and that targeting this MMP might be beneficial to a subset of patients with IPF who show sufficient expression of type 1 IFNs.

Mujahed T. Malignant Peritoneal Mesothelioma Arising in Young Adults With Long-standing Indwelling Intra-abdominal Shunt Catheters. Am J Surg Pathol. 2021; 45(2):255-262.

- Only 50% to 70% of patients with mesothelioma report asbestos exposure while some patients have no obvious cause.
- The authors described a series of patients with long-standing indwelling intra-abdominal shunt catheters who developed malignant peritoneal mesothelioma, suggesting a novel association.
- 7 patients who had shunts and subsequently developed mesothelioma (5 women; median age: 31 y, range: 18 to 45 y).
- 6 patients had hydrocephalus and a ventriculoperitoneal shunt, and 1 patient had portal hypertension and a portoatrial shunt. The median duration of shunt therapy in 5 cases was 29 years (range: 12 to 35 y); the remaining 2 patients also had shunts for many years, but specific details were unavailable. Two patients had radiotherapy for malignancies in childhood. One had an alleged exposure to asbestos and 1 had prior exposure to talc. The rest had no known risk factors.
- Histologically, all tumors were purely epithelioid.
- All 7 died of disease (median survival: 7 mo, range: 1 to 18 mo).
- Molecular testing showed loss of NF2 and CDKN2A/B and a BAP1 mutation in 1 case, and no genomic alterations associated with mesothelioma in 2 cases.
- Peritoneal mesothelioma may represent a complication of long-standing indwelling shunt catheters. The mechanism is unknown, but chronic peritoneal irritation may play a role.

Ren HZ. Pleural Malignant Mesotheliomas Do Not Demonstrate SWItch/Sucrose Nonfermentable (SWI/SNF) Complex Deficiency. Am J Surg Pathol. 2021; 45(2):290-291. and Loarer FL. Response To: Pleural Malignant Mesotheliomas Do Not Demonstrate

SWitch/Sucrose Nonfermentable (SWI/SNF) Complex Deficiency. Am J Surg Pathol. 2021; 45(2):291-292.

- Since malignant mesotheliomas can show some morphologic and clinical overlap with SMARCA4-deficient thoracic sarcomas, the suggestion from Perret and colleagues that a significant portion of mesotheliomas lose SMARCA4 deserves further examination.
- Perret and colleagues identified loss of SMARCA4 expression in 4/31 (13%) epithelioid mesotheliomas, while in their series, Ahadi and Gill identified only 2 of 296 (0.7%) mesotheliomas showing SMARCA4 loss, also both of epithelioid type.
- In the cohort from UBC, no case showed the loss of any core SWI/SNF protein. The analysis of this independent cohort thus did not demonstrate loss of any core SWI/SNF proteins, including SMARCA4, in pleural mesotheliomas.
- These findings raise a question of whether the few cases reported as mesotheliomas showing loss of SMARCA4 were really mesotheliomas.
- In retrospect, all but 1 SMARCA4-lost “mesotheliomas” had indeed an equivocal phenotype with only partial and isolated expression of a subset of mesothelioma markers, confirming the Ahadi and Gill finding that the subset of the so-called mesotheliomas associated with SMARCA4 loss may actually represent poorly differentiated tumors rather than straightforward mesotheliomas.

Rodic N. Positive Postmortem Test for SARS-CoV-2 Following Embalming in Confirmed COVID-19 Autopsy. Am J Clin Pathol. 2021; 155(2):318-320.

- A nasopharyngeal swab and a lung swab of the left lower lobe were sent to a reference laboratory for the severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) nucleic acid amplification test.
- The COVID-19 test yielded positive results following the embalming procedure (vascular phase entailed injection of 22 L solution of 2.4% formaldehyde, and visceral phase entailed injection of 11 L solution of 3% formaldehyde; both solutions also contained accessory fluids: methanol, monopropylene glycol, and mineral salts).
- Based on this empirical observation, the case serves as proof-of principle that embalming is not an absolute contraindication for COVID-19 testing.
- Moreover, thought can be given to adding formaldehyde to collection kits, which may preclude or minimize chance of exposure to laboratory technicians during preanalytical specimen handling and procurement.

Shibata Y. Evaluation of variant frequency in SARS-CoV-2 infection-related genes utilizing lung cancer genomic database. Lung Cancer. 2021; 152:199-201.

- The numbers of cases of and deaths from COVID-19 in East Asian countries are obviously lower than those in other parts of the world.
- Angiotensin-converting enzyme 2 (*ACE2*) and transmembrane serine protease 2 (*TMPRSS2*) are crucial for SARS-CoV-2 infection. Recent studies have indicated that the genetic diversity of *ACE2* and *TMPRSS2* variants across races might affect these functions and the susceptibility, symptoms and outcome of SARS-CoV-2 infection.
- Authors analyzed the coding variants of *ACE2* and *TMPRSS2* and their allele frequencies (AFs) in 350 Japanese patients with advanced-stage lung cancer using whole-exome sequencing data in East Asia (LC-SCRUM-Asia). Then the authors compared the observed genetic variants and their AFs with those of other Japanese cohorts and other ethnic populations using publicly available genomic databases: dbSNP, GnomAD and TOPMed.
- In the Japanese lung cancer cohort, 9 and 7 variants were found in *ACE2* and *TMPRSS2*, respectively. The AFs of c. 439 + 4G > A in *ACE2* (rs2285666) and c.589 G > A in *TMPRSS2* (rs12329760) were much higher in East Asians (0.5281 and 0.4172, respectively) in both our cohort and another Japanese cohort (The Tohoku Medical Megabank Project database) than in Caucasians (0.3692 and 0.1722, respectively).
- c.23 G > T in *TMPRSS2* (rs75603675) was more frequent in Caucasians than in East Asians (0.2845 vs. 0.0141, respectively).
- The above-mentioned variants with significant AF differences might be associated with the differences in disease severity of COVID-19 between East Asians and Caucasians.

Chang W-C. Mucinous adenocarcinoma arising in congenital pulmonary airway malformation: clinicopathological analysis of 37 cases. *Histopathology*. 2021; 78(3):434-444.

- Mucinous adenocarcinoma arising in congenital pulmonary airway malformation (CPAM) is a rare complication.
- Thirty-seven cases were collected within a 34-year period, and the subtype of adenocarcinoma and CPAM, tumor location, stage, growth patterns, molecular data and follow-up were recorded.
- The cohort comprised CPAM type 1 (n = 33) and CPAM type 2 (n = 4). Morphologically, 34 cases were mucinous adenocarcinomas (21 in situ; 13 invasive), and three were mixed mucinous and non-mucinous adenocarcinoma.
- Seventeen cases showed purely extracystic (intra-alveolar) adenocarcinoma, 15 were mixed intracystic and extracystic, and five showed purely intracystic proliferation.
- Genetically, nine of 10 cases tested positive for KRAS mutations, four with exon 2 G12V mutation and five with exon 2 G12D mutation.
- Residual disease on completion lobectomy was observed in two cases, and three cases recurred 7, 15 and 32 years after the original diagnosis.

- Two patients died of metastatic invasive mucinous adenocarcinoma.
- Most adenocarcinoma that arise in type 1 CPAMs, are purely mucinous, and are early-stage disease.
- Intracystic proliferation is associated with lepidic growth, an absence of invasion, and indolent behaviour, whereas extracystic proliferation may be associated with more aggressive behaviour and advanced stage.
- Most cases are cured by lobectomy, and recurrence/residual disease seems to be associated with limited surgery. Long-term follow-up is needed, as recurrence can occur decades later.

Liang P. A possible EGFR TKIs resistance mechanism of lung adenocarcinoma patients with a novel EGFR exon 20-ins mutation: A case report. Lung Cancer. 2021; 152:193-195.

- Approximately 4–12 % of patients with NSCLC harbor EGFR exon20 insertion mutations and the majority (except for a few subtypes such as EGFR A763_Y764insFQEA) are associated with poor responses with 1st and 2nd generation EGFR-tyrosine kinase inhibitors (TKIs).
- The patient was a 64-year-old female non-smoker with a hard lump in the apical posterior segment of the left superior lobe, which was considered to be peripheral lung cancer, and multiple metastases in the left lung, and multiple lymph nodes in the hilar area and mediastinum.
- The patient was initially treated with osimertinib and displayed stable disease treatment to the targeted therapy after two months. After progressive pleural effusion, gefitinib was administered to the patient.
- EGFR P772delinsVDNR, a novel EGFR exon20 insertion mutation, was identified.
- This case showed a possible mechanism for resistance to 1st and 3rd EGFR TKIs. It should be noted that the sensitivity of patients harboring this mutation to second-generation EGFR TKI remains to be explored.

Hopfer H. Hunting coronavirus by transmission electron microscopy – a guide to SARS-CoV-2-associated ultrastructural pathology in COVID-19 tissues. Histopathology. 2021; 78(3):358-370.

- Transmission electron microscopy has become a valuable tool to investigate tissues of COVID-19 patients because it allows visualization of SARS-CoV-2, but the 'virus-like particles' described in several organs have been highly contested.
- Using micrographs from infected cell cultures and autopsy tissues, the authors showed how coronavirus replication affects ultrastructure and put the morphological findings in the context of viral replication, which induces extensive remodeling of the intracellular membrane systems.

- Virions assemble by budding into the endoplasmic reticulum-Golgi intermediate complex and are characterized by electron-dense dots of cross-sections of the nucleocapsid inside the viral particles. Physiological mimickers such as multivesicular bodies or coated vesicles serve as perfect decoys.
- Compared to other in-situ techniques, transmission electron microscopy is the only method to visualize assembled virions in tissues and will be required to prove SARS-CoV-2 replication outside the respiratory tract.
- In practice, documenting in tissues the characteristic features seen in infected cell cultures seems to be much more difficult than anticipated. The hunt for coronavirus by transmission electron microscopy is still on.

Deshmukh V. Histopathological observations in COVID-19: a systematic review. J Clin Pathol. 2021; 74(2):76-83.

- Data on pathological changes in different organs are still scarce, the authors aimed to review and summarize the latest histopathological changes in different organs observed after autopsy of COVID-19 cases.
- Over the period of 3 months, authors performed vast review of the articles. The search engines included were PubMed, Medline (EBSCO & Ovid), Google Scholar, Science Direct, Scopus and Bio-Medical. Search terms used were ‘Histopathology in COVID-19’, ‘COVID-19’, ‘Pathological changes in different organs in COVID-19’ or ‘SARS-CoV-2’. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 guidelines were used for review writing.
- The authors identified various articles related to the histopathology of various organs in COVID-19 positive patients.
- Overall, 45 articles were identified as full articles to be included in our study. Histopathological findings observed are summarized according to the systems involved.
- In lung:
 - Alveoli: Damaged or atypical enlarged pneumocytes with large nuclei, type II pneumocyte hyperplasia, diffuse alveolar damage (DAD), focal sloughing, hyaline membrane formation, intra-alveolar hemorrhage, intra-alveolar neutrophil infiltration, amphophilic granular cytoplasm and prominent nucleoli characteristic of viral cytopathic-like changes.
 - Vessels: Edematous and congested vessels, plug formation, fibrinoid necrosis of the small vasculature, hyaline thrombi in microvessels. Significant deposits of complements—C5b-9 (membrane attack complex), C4d, and mannose binding lectin (MBL)-associated serine protease (MASP)-2, in the microvasculature.

- Cellular components: Presence of syncytial giant cells, focal infiltration of immune and inflammatory (lymphocytes and monocytes) and increased stromal cells.
- Ultrastructural changes: Viral particles in bronchial mucosal epithelia and type II alveolar epithelia.
- Although COVID-19 mainly affects respiratory and immune systems, but other systems like cardiovascular, urinary, gastrointestinal tract, reproductive system, nervous system and integumentary system are not spared, especially in elderly cases and those with comorbidity.

Kohli M. Diagnostic accuracy of centralised assays for TB detection and detection of resistance to rifampicin and isoniazid: a systematic review and meta-analysis. Eur Respir J. 2021; 57(2):2000747

- Various diagnostic companies have developed high throughput molecular assays for tuberculosis (TB) and resistance detection for rifampicin and isoniazid.
- The authors performed a systematic review and meta-analyses to assess the diagnostic accuracy of five of these tests for pulmonary specimens.
- The tests included were Abbott RealTime MTB, Abbott RealTime RIF/INH, FluoroType MTB, FluoroType MTDBR and BD Max MDR-TB assay.
- A comprehensive search of six databases for relevant citations was performed. Cross-sectional, case-control, cohort studies, and randomized controlled trials of any of the index tests were included. Respiratory specimens (such as sputum, bronchoalveolar lavage, tracheal aspirate, *etc.*) or their culture isolates.
- A total of 21 included studies contributed 26 datasets.
- For TB detection, the included assays had a sensitivity of 91% or more and the specificity ranged from 97% to 100%.
- For rifampicin resistance detection, all the included assays had a sensitivity of more than 92%, with a specificity of 99–100%.
- Sensitivity for isoniazid resistance detection varied from 70 to 91%, with higher specificity of 99–100% across all index tests.
- Studies that included head-to-head comparisons of these assays with Xpert MTB/RIF for detection of TB and rifampicin resistance suggested comparable diagnostic accuracy.
- In people with symptoms of pulmonary TB, the centralised molecular assays demonstrate comparable diagnostic accuracy for detection of TB, rifampicin and isoniazid resistance to Xpert MTB/RIF assay, a WHO recommended molecular test.

Hwang B. Potential role of exosome-based allorecognition pathways involved in lung transplant rejection. J Thorac Cardiovasc Surg. 2021; 161(2):e129-e134.

- Innate and adaptive immunity both contribute to allorecognition mechanisms that drive rejection after lung transplantation.

- Classic allorecognition pathways have been extensively described, but there continues to be several unanswered questions.
- Exosomes are extracellular vesicles involved in a multitude of immune responses and are capable of eliciting and potentiating innate and adaptive immunity in lung transplant rejection.
- Exosome-mediated allorecognition involves both innate and adaptive immunity.
- There have been numerous studies investigating the roles of direct, indirect, and semi-direct pathways in allorecognition driving transplant rejection.
- Further studies investigating donor or host-derived exosomes locally or in the secondary lymphoid organs in exosome-mediated allorecognition may provide new perspectives in addressing outstanding questions in the area of transplantation.
- The addition of potential exo-allo-recognition pathways to the conventional theories provides significant insight into other mechanisms contributing to donor and recipient alloreactivity.
- The clearance of donor passenger leukocytes, or inability to migrate to recipient lymphoid tissues, suggests that donor graft-derived exosomes play a significant role in alloantigen recognition pathways.
- Although the mechanisms in which exosomes mediate acute and chronic rejection are still unclear, further studies investigating these pathways are pivotal to understanding the development of these immune processes.