

Pathology of Lung Transplantation

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Major topics in this presentation

- Pathologic grading of rejection:
The new ISHLT classification system.
- Antibody-mediated rejection
- The pathogenesis of obliterative bronchiolitis/chronic rejection.

Lung Transplant Pathology

Yousem SA, et al., Revision of the 1990 Working Formulation for the Classification of Pulmonary Allograft Rejection: Lung Rejection Study Group.

The Journal of Heart and Lung Transplantation 1996; 15: 1-15.

Lung Transplant Pathology

Stewart S, et al., Revision of the 1995 working formulation for the standardisation of nomenclature in the diagnosis of lung rejection

(In Press; *The Journal of Heart and Lung Transplantation*)

Lung Transplant Pathology

- The diagnosis of rejection is one of exclusion; infection and other lesions must be ruled out.
- At least 5 pieces of alveolar parenchyma are necessary for a reliable diagnosis.
- At least 3 h&e stained slides from 3 levels in the block and trichrome (connective tissue) stain must be reviewed.

Lung Transplant Pathology: Revised ISHLT grading

- | | |
|---------------------------|--|
| <i>A. Acute Rejection</i> | <i>B. Airway Inflammation (Bronchioles only)</i> |
| 0 - None | 0 - None |
| 1 - Minimal | <u>1R</u> - Low grade |
| 2 - Mild | <u>2R</u> - High grade |
| 3 - Moderate | X - Ungradeable |
| 4 - Severe | |
| X - Ungradeable | |

Acute Lung Rejection

- A0 No infiltrates
- A1 Rare circumferential perivascular infiltrates, 2-3 cells thick ; no eosinophils or endothelialitis.
- A2 Perivascular infiltrates readily seen at low magnification; eosinophils and endothelialitis are frequent.
- A3 Infiltrates extend into alveolar septae.
- A4 Diffuse infiltrates and alveolar injury.
- AX Fewer than 5 good pieces of alveolar parenchyma

Infection in the Lung Allograft

- FUNGAL: Candida, Aspergillus, Cryptococcus
- VIRAL: CMV, Adenovirus
- Pneumocystis carinii: Granulomatous; cysts may be very rare
- BACTERIAL

Other pathology in the lung graft

- Infection
- Reperfusion injury
- Aspiration pneumonia
- Allergic Bronchopulmonary Aspergillosis
- Bronchiolitis Obliterans - Organizing Pneumonia
- Bronchus-associated lymphoid tissue

Other pathology in the lung graft (continued)

- Drug toxicity (Rapamycin – org. pneumonia)
- Post-transplant lymphoproliferative disease
- Biopsy sites
- Recurrent native disease: Sarcoid, Langerhans cell histiocytosis, lymphangioleiomyomatosis, BAC, DIP.

Acute Lung Rejection: Airway Inflammation (Bronchioles only)

- B0** – No bronchiolar inflammation
- B1R** (low grade small airway inflammation) – Submucosal mononuclear cells with occasional eosinophils. May be circumferential. No epithelial damage or intraepithelial infiltration.
- B2R** (high grade small airway inflammation) - Eosinophils and plasmacytoid cells present with intra-epithelial inflammation and epithelial necrosis.
- BX** - Ungradeable

Chronic Lung Rejection: Airways and Vessels

- C. Chronic Airway Rejection – Obliterative Bronchiolitis**
- C0: No obliterative bronchiolitis**
- C1: Obliterative bronchiolitis is present**
- D. Chronic Vascular Rejection - Accelerated Graft Vascular Sclerosis (Arteries and/or veins)**

Is there humoral rejection in lung transplants?

- Hyperacute rejection not yet defined.
- Humoral rejection not yet defined.

Saint Martin GA, Reddy VB, Garrity ER, et al. Humoral (Antibody-mediated) Rejection in Lung Transplantation. J Heart Lung Transplant 1996; 15: 1217-22.

No IgG, IgM or C3c demonstrated in vessels, alveoli or interstitium in 90 biopsies from 55 patients.

Stages of humoral response to an organ graft

- I. Latent – Circulating antibody (to HLA or other endothelial antigens)
- II. Silent – Circulating antibody + C4d deposition
- III. Subclinical – Circulating antibody + C4d + tissue pathology
- IV. Humoral rejection – Circulating antibody + C4d + tissue pathology + graft dysfunction

Takemoto, et al., National conference to assess antibody-mediated rejection in solid organ transplantation. Am J Transplant 2004; 4: 1033-41.

Lung: Humoral rejection

Circulating anti-HLA and patchy C4d deposition in graft with low sensitivity and low specificity.

Ionescu DN, Girmita AL, Zeevi A, et al. C4d deposition in lung allografts is associated with circulating anti-HLA antibody. Transpl Immunol 2005; 15:63-8.

Sensitized patients have more post-tx ventilator days than do non-sensitized patients.

Lau Cl, Palmer SM, Posther KE, et al., Influence of panel-reactive antibodies on posttransplant outcomes in lung transplant recipients. Ann Thorac Surg 2000; 69: 1520-4.

Lung: Humoral rejection

C4d deposition is a stronger predictor of septal capillary necrosis and clinical acute rejection than are C1q, C5b-9, or Ig.

C4d and C1q are deposited in bronchial walls in Bronchiolitis Obliterans Syndrome.

Magro CM, Pope Harmon A, Klinger D, et al. Use of C4d as a diagnostic adjunct in lung allograft biopsies, Am J Transplant 2003; 3: 1143-54.

Lung: Humoral rejection

C4d staining may be positive in variable and non-specific patterns.

WallaceWD, Reed EF, Ross D, Lassman CR, Fishbein MC. C4d staining of pulmonary allograft biopsies: an immunoperoxidase study. J Heart Lung Transplant 2005; 24: 1565-70.

Bronchiolitis Obliterans

- Toxic fumes
- Respiratory infections
- Connective tissue disorders
- Following bone marrow or lung transplantation

Post-transplant Obliterative Bronchiolitis

- 50 – 60% of patients surviving 5 years.
- Median time to diagnosis is 16 – 20 months.
- Bronchiolitis Obliterans Syndrome (BOS):
A clinical classification based on % decrease in FEV-1 and FEV 25-75 compared with baseline.

Cooper JD, et al., J Heart Lung Transplant 193; 12: 713-716.

OB: Alloimmune-dependent factors

- Acute rejection, particularly if high grade or persistent or late-onset.
- ?Lymphocytic bronchitis/bronchiolitis
- HLA mismatch
- Development of anti-HLA antibodies

OB: Alloimmune-independent factors

- Cytomegalovirus infection
- Other lung infections (RSV, parainfluenza, influenza, adenovirus, rhinovirus)
- Chemical injury from aspiration with gastroesophageal reflux disease

OB: Alloimmune-independent factors

- Trigger the innate immune system (PMN, monocytes, eosinophils, NK cells, cytotoxic cells, dendritic cells) via Toll-like receptors.
- Hyporesponsiveness with polymorphisms for TLR-4 receptor (Asp299Gly or Thr399I11) leads to decreased rates of acute rejection and BOS after lung transplantation.

Innate immunity is linked with adaptive immunity.

OB: Cells

- T-cells
- Neutrophils
- Monocytes/macrophage
- Fibroblasts & endothelial cells

Murine heterotopic tracheal transplant model:

T-cells required (CD8 > CD4); B-cells play a minor role; neutrophils are not required.

Cautions: This model is not a functional airway and is not primarily vascularized and human OB is primarily a disease of small airways.

OB: Cytokines and Chemokines

- T-cell growth factors – IL2, TNF α , β , IFN γ , IL-12, IL-6.
- Chemokines – CCL2, CXCL2, 10, 11, CXCR2, RANTES.
- Cytolytic effectors – perforin, granzyme.
- Remodeling – matrix metalloproteinases, ET-1, PDGF, FGF, IGF-1, TGF- β

Post-transplant Obliterative Bronchiolitis

A fibro-obliterative response to alloimmune factors and non-immune factors engaging both the adaptive and innate immune systems.

The Pulmonary Pathology of Iatrogenic Immunosuppression

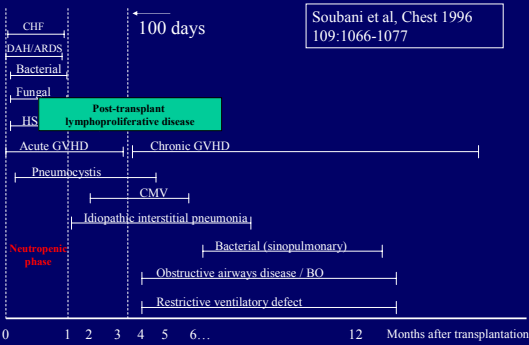
Kevin O. Leslie, M.D.
Mayo Clinic Scottsdale



The indications for iatrogenic immunosuppression

- Autoimmune/inflammatory disease
- Chemotherapy for malignant neoplasm
- Bone marrow and solid organ transplantation

Lung Disease Timetable Post BMT



The spectrum of subsequent disease

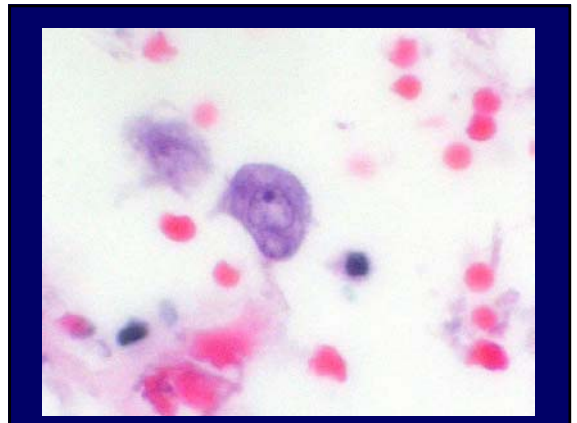
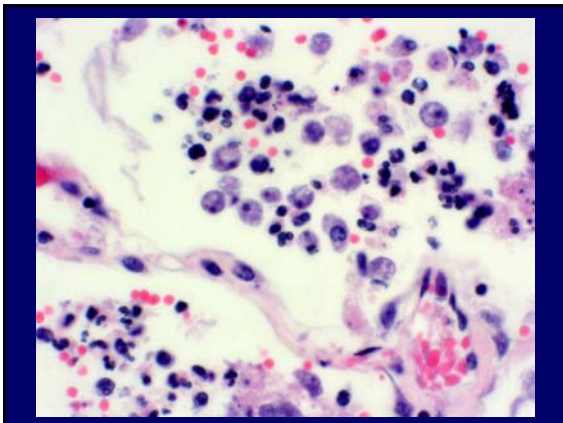
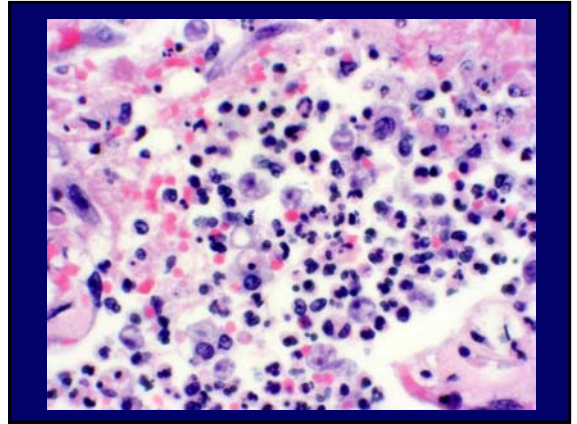
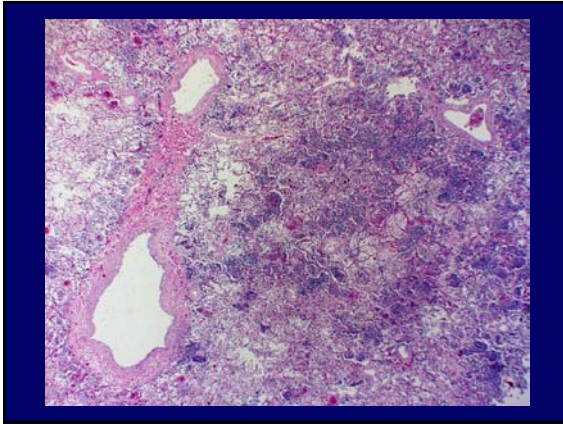
- **Infection**
- Therapy-related lung disease
- Recurrence of original disease
- Graft versus host disease
- Post immunosuppression immunoproliferative disease
- Transplant rejection

A 58 year old woman renal transplant recipient presented with symptoms of acute pneumonia and meningoencephalitis.

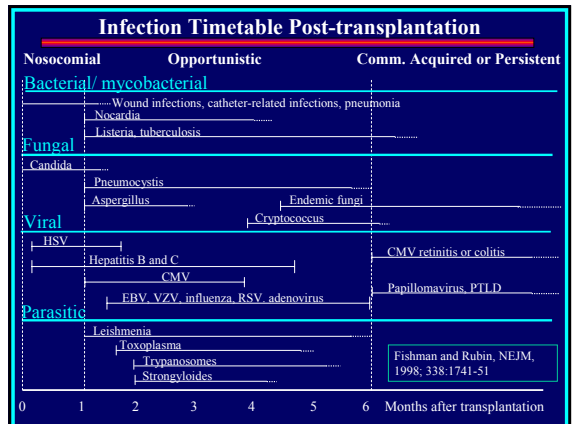
Chest imaging showed diffuse bilateral infiltrates. Bronchoscopic evaluation was terminated before biopsy, and bronchial washings failed to grow any organisms.

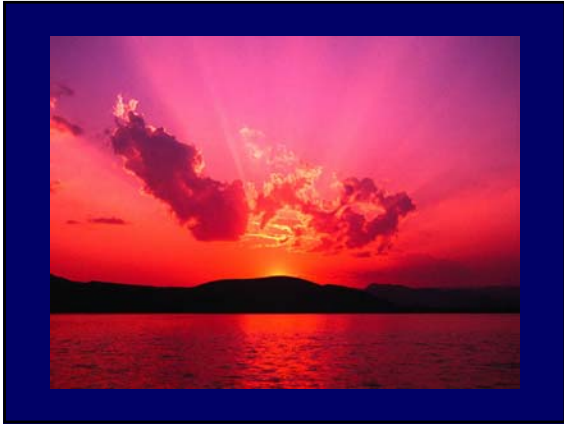
Despite broad spectrum antibiotics and intensive support, she expired several days after admission.

He was treated with aerosolized ribavirin, but his infiltrates progressed. A surgical lung biopsy was performed.



Diagnosis
 Category 1 Infection
**Acute Pneumonia and
 Meningoencephalitis caused by
 Free-living Amoebae**
Acanthamoeba spp. Vs *Balamuthia mandrillaris*
 Int J Parasitol. 2004 Aug;34(9):1001-27.
 Rev Pneumol Clin. 1998 Dec;54(6):346-52.
 Clin Chest Med. 2002 Jun;23(2):479-92





The spectrum of subsequent disease

- Infection
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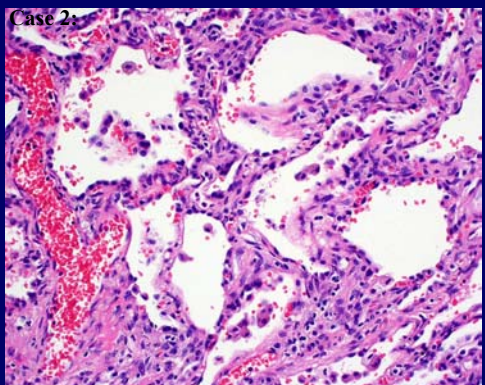
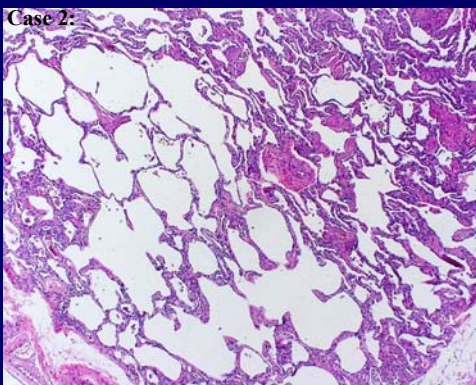


A 68 year old woman presented with breathlessness and hypoxemia. Five months earlier she had been diagnosed with large B cell lymphoma (CD20 positive) and was treated with decadron and Rituxan.

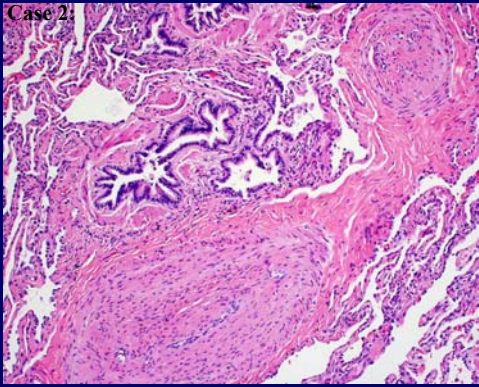
One month after beginning therapy, she complained of cough and shortness of breath. She was found to be neutropenic and was begun on antibiotic therapy (vancomycin) for presumed pneumonia.

Her neutropenia improved, but her cough and breathlessness progressed. She was begun on azithromycin and bronchodilators without improvement.

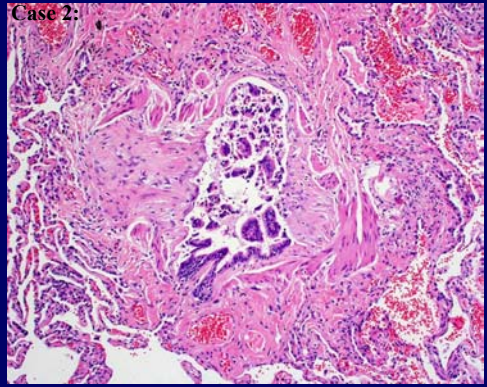
A surgical lung biopsy was performed.



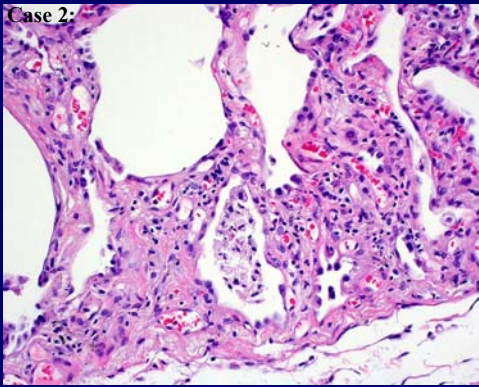
Case 2:



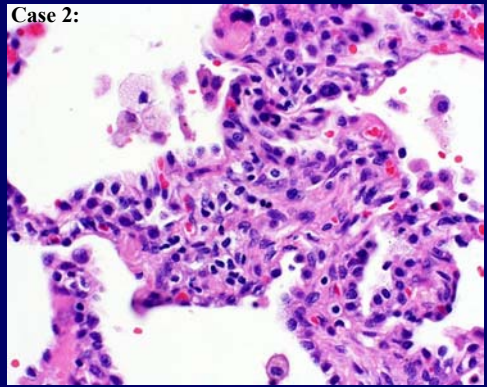
Case 2:



Case 2:



Case 2:



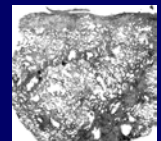
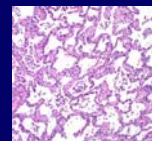
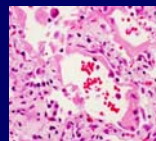
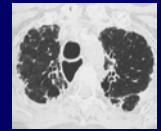
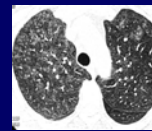
Diagnosis

Category 2: Therapy-associated disease

Cellular interstitial pneumonia with small airways disease and evidence of constrictive bronchiolitis. There are subacute arteriopathic changes possibly related to therapy.

The favored interpretation for the patient's interstitial pneumonia is drug effect related to Retuxin therapy.

3 Main Patterns of Drug Reaction



ALI/EP-like

NSIP/HSP-like

Fibrosis

Rituximab is a murine monoclonal antibody directed against CD20. FDA indications include relapsed (or refractory) low grade or follicular, CD20 positive B cell lymphomas. May be used in combination therapy with other agents in higher grade CD20 positive lymphomas.

Pulmonary toxicity related to rituximab is reported but uncommon.

Interstitial pneumonia associated with rituximab may be severe.

Jullien V, et al. Rev Mal Respir. 2004 Apr;21(2 Pt 1):407-10



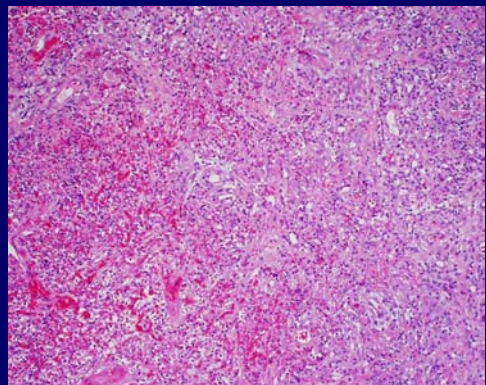
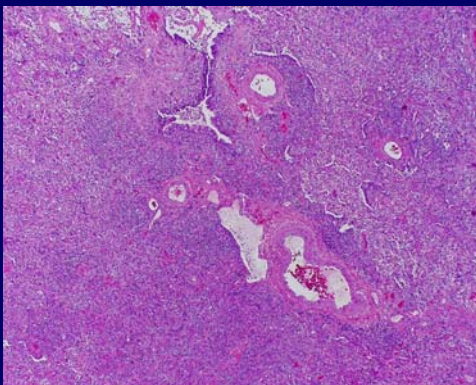
The spectrum of subsequent disease

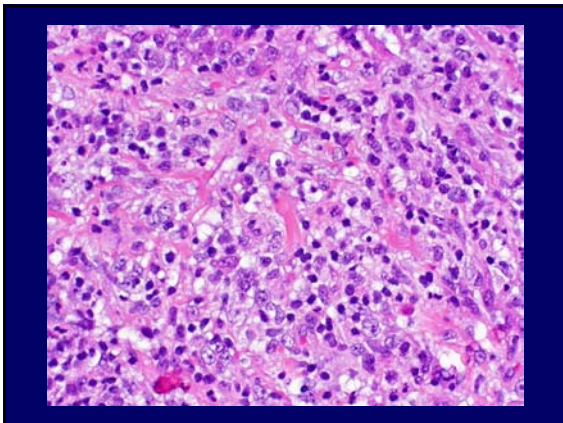
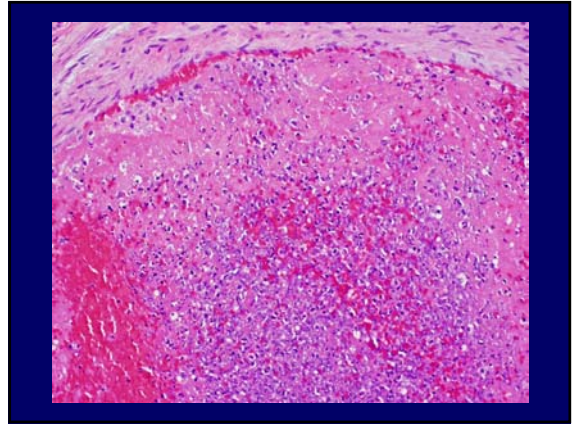
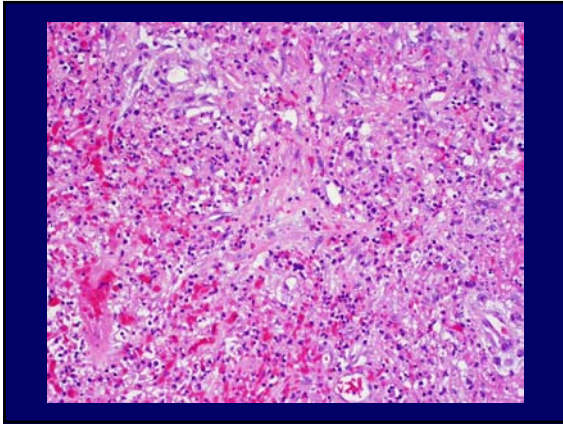
- Infection
- Therapy-related lung disease
- **Recurrence of original disease**
- Graft versus host disease
- Post immunosuppression immunoproliferative disease
- Transplant rejection



A 58 year old woman presented with progressive respiratory difficulty and reticulonodular infiltrates on CT scan. The past medical history is remarkable for nodal large B cell lymphoma (CD20/30+) with extensive bone marrow involvement two years earlier.

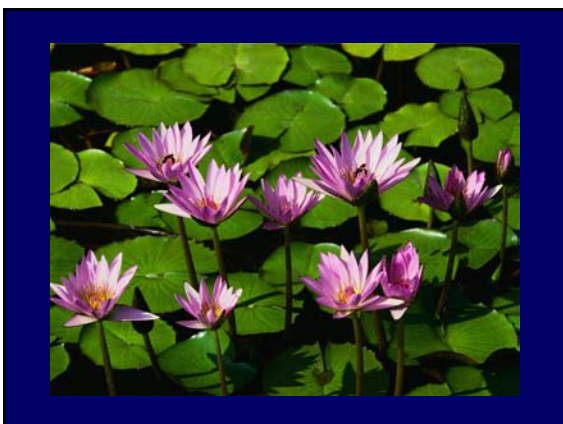
The patient had been receiving chlorambucil for several months before her current presentation. A surgical lung biopsy was performed.




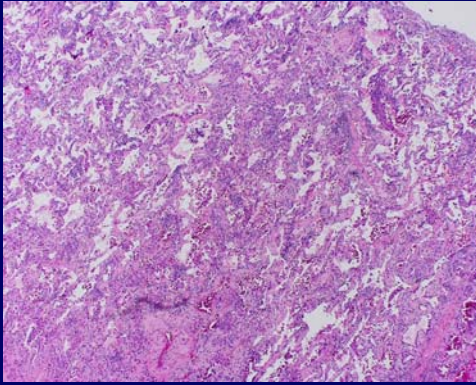


Diagnosis
Category 3: Recurrent disease

Recurrent large B cell
lymphoma



- The spectrum of subsequent disease
- Infection
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- 

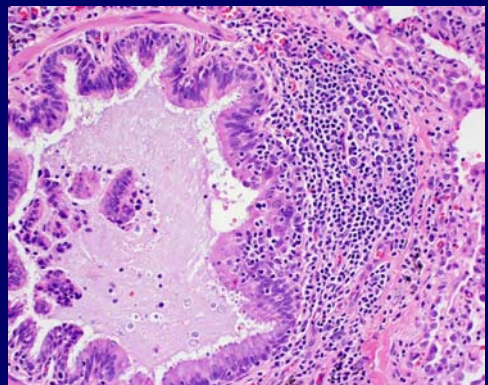
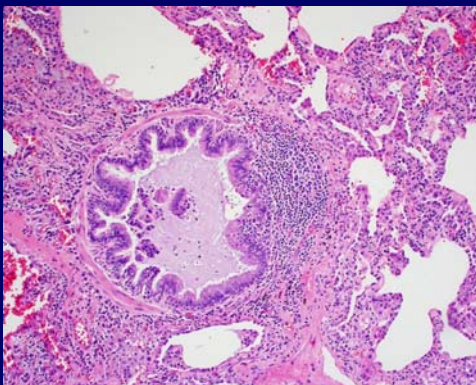
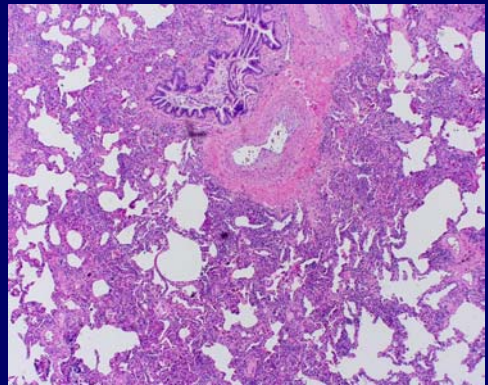


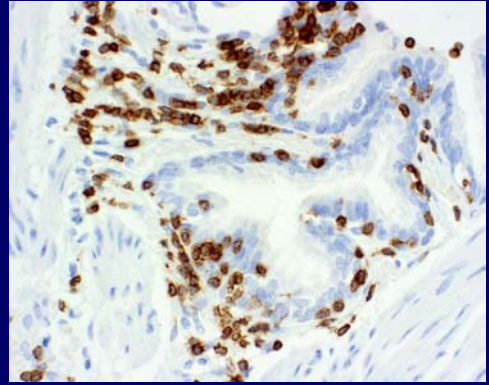
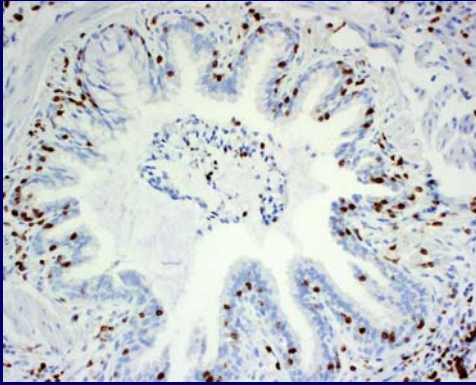
A 43 year old man presented with progressive shortness of breath and radiologic changes suggesting pulmonary fibrosis.

The past medical history is remarkable for CML diagnosed 7 years earlier and treated with allogeneic bone marrow transplantation from a living related donor.

The intervening years were punctuated by CNS toxoplasmosis and graft-versus-host disease (skin and GI tract). The FEV1 on admission was 46%. The patient also complained of decreased exercise tolerance and unintentional weightloss.

A surgical lung biopsy was performed.





Diagnosis

Category 4: GVHD

Pulmonary graft versus host disease

Pulmonary Graft versus Host Disease

Spectrum of Pathologic Manifestations

1. Idiopathic pneumonia syndrome (IPS)/ LIP
2. Lymphocytic bronchiolitis
3. Perivascular lymphocyte cuffing (mature T cells)
4. Pulmonary cytolytic thrombi
5. Bronchiolitis obliterans (constrictive bronchiolitis)
6. Advanced pulmonary fibrosis

Pulmonary Graft versus Host Disease

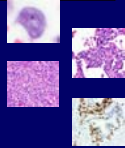
CT findings (> 100 days post transplant)

1. Patchy consolidation and ground glass attenuation
2. Bronchial dilatation
3. Mosaic air-trapping



The spectrum of subsequent disease

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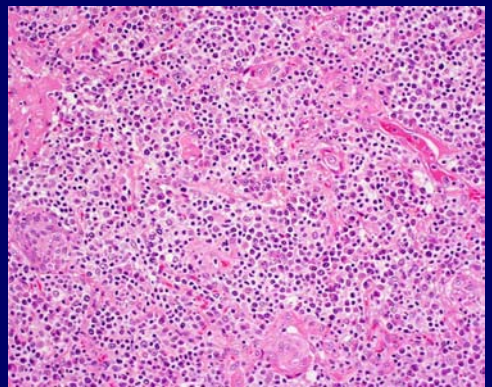
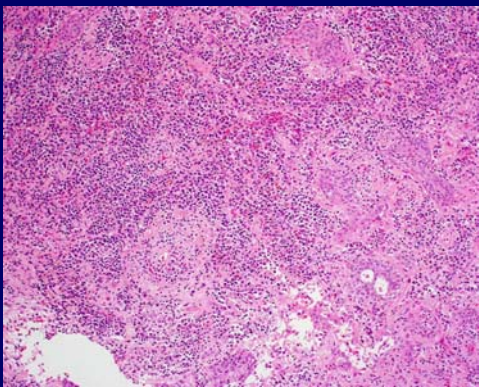
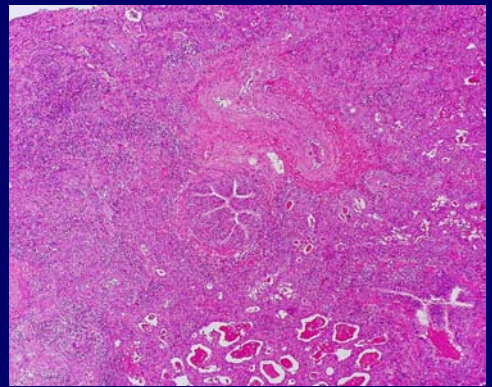


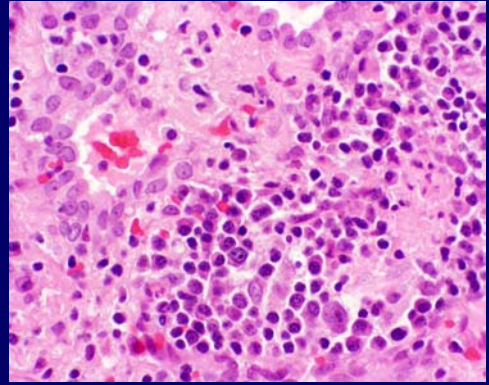
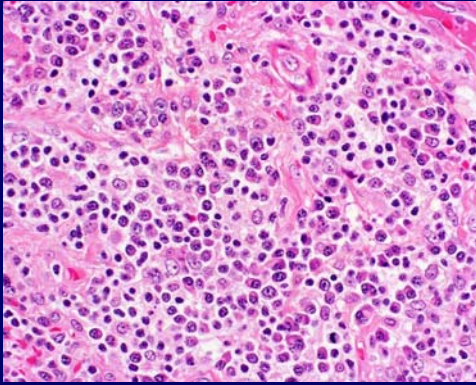
A 59 year old man presented with shortness of breath and fatigue. He was 1 month status post his second allogeneic bone marrow transplant for recurrent multiple myeloma. Two weeks prior to admission, a nasal swab was positive for RSV.

Chest imaging initially revealed a slightly nodular peripheral infiltrate and this progressed over the subsequent three weeks to involve predominately mid and lower lung zones bilaterally, with minimal upper lobe infiltrates.

He was treated with aerosolized ribavirin, but his infiltrates progressed.

A surgical lung biopsy was performed.





Diagnosis

Category 5 PILPD/PTLPD

Epstein-Barr virus-associated post-transplant polyclonal lymphoproliferative disorder (EBV-PTLD).

A component of GVHD is present and there are extensive airway reparative changes (squamous metaplasia). No RSV viropathic changes seen.

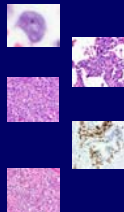
Multiple pulmonary nodules caused by B-cell post-transplant lymphoproliferative disorder after bone marrow transplantation: monitoring Epstein-Barr virus viral load.

Shoji N, Ohyashiki JH, Suzuki A, Kubota N, Kimura Y, Matsubayashi J, Mukai K, Ohyashiki K.

Jpn J Clin Oncol. 2003 Aug;33(8):408-12.

The 5 Take Home Lessons

1. Infection
2. Therapy-related lung disease
3. Recurrence of original disease
4. Post immunosuppression immunoproliferative disease
5. Graft versus host disease



Trying to Manage the Unmanageable: The Lung Transplant Clinical “Protocol”
(Goal: decrease patient complications without increasing the clinician’s anxiety)

Underlying Theme: Lung Transplant Problems are Ubiquitous and Nonspecific

The pensive/worried pulmonary clinician talking to you about a lung transplant problem:

- “It could be anything!”
- “It all looks the same to me”
- “That’s why we got the biopsies!”
- “We trust your judgment”
- “The patient’s outcome is in your hands”

Surgeon’s response to discussion: “\$%&#!!!! clinicians”

In patient Hospitalization in the Post Operative Period (usually ~2 weeks)

Possible Major Lung Complications

1. **Ischemia Reperfusion Injury (I/R: ARDS/DAD):** usually diagnosed on clinical grounds with hypoxemia (low PaO2/FiO2 ratio), diffuse symmetric “edema” pattern on chest xray, noncompliant lungs physiologically. In general this problem has decreased in magnitude due to better preservation solutions and surgical techniques. The majority of patients recover but nonetheless severe forms can lead to death or other complications.
2. **Infections:** Usually bacterial (nosocomial organisms). Present with pulmonary infiltrates of any nature, cough sometimes, fever, leukocytosis. Diagnosis usually made by culture of lung secretions. Most patients respond well to antibiotics. This problem too has declined somewhat due to intensive antimicrobial regimens used prophylactically.
3. **Acute Allograft Rejection:** Presentation not dissimilar to the above problems and potentially overlapping. Dx can be empiric but most prefer pathologic confirmation with Transbronchial biopsy (TbBx). Rx: High dose steroids which is effective in the vast majority.
4. **Airway Dehiscence:** Usually in the first few weeks. Less common (<5%) in the modern era. Dx: clinical. Most heal spontaneously but some require surgery.
5. **Hyperacute Rejection:** From preformed antibodies. Very rare in the modern era given recipient alloantibody evaluation by PRA and flow cytometric methods.

Medical Management: the First Year

Surveillance

1. Daily temperatures, BPs, spirometry (hand held) and symptom diary.
2. Thrice weekly: exercise tolerance and oxyhemoglobin saturation during rehabilitation
3. Weekly to monthly clinic visits for symptom review, exams, lab work (including CMV monitoring with viral load by PCR), chest xrays and formal spirometry.
4. Quarterly bronchoscopies (programs vary) looking mostly for silent AR or occult infection.
5. Preventative medications: immunosuppressants, PCP/CMV/fungi/herpes prophylaxis (varying protocols)

Possible Major Lung Complications

1. **Infections:**

(A) Usually bacterial or fungal (mix of nosocomial and community acquired organisms).

Presentation usually patchy infiltrates, often bilateral accompanied by fever, leukocytosis and cough. Diagnosis by sputum/bronchoscopy culture.

(B) CMV also occurs in this period as preventative medications (valganciclovir) are withdrawn. Patients present most commonly with fatigue and leukopenia with or without respiratory symptoms. Infiltrates, if present, are diffuse and symmetric but most cases are diagnosed before infiltrates arise. Diagnosis: Gold standard is pathology but most centers initiate treatment with a positive PCR result (the sensitivity and specificity of this test is still be evaluated in clinical research studies).

(C) **Fungal Infections:** less common. Typically more nodular in appearance or cavitating. Diagnosis by culture of secretions, rarely by pathology.

(D) **Other infections:** rarely Legionella, Nocardia, NTM, more commonly community viruses. Diagnosis rests on stains, cultures and probes.

2. **Acute Vascular Allograft Rejection:** Often chest xray silent. Symptoms range from none to dyspnea and/or cough and/or fever. Often detected early with declines in spirometric indices or with surveillance bronchoscopy in a “well” patient. Dx can be empiric but most prefer pathologic confirmation with TbBx. The ISHLT severity scoring system is universally used. Rx: High dose steroids which is effective in the vast majority.
3. **Humoral Allograft Rejection:** May present subtly or fulminantly with hypoxia, diffuse infiltrates, respiratory failure. May mimic ARDS from sepsis but shock is usually not present. Dx clues: timing and presentation, bloody BAL fluid, HLA antibodies in serum (although certainly not critical). Pathology is gold standard with capillaritis and C4d staining (but much needs to be worked out regarding the sensitivity and specificity of the C4d stain). Rx: Plasma Exchange, steroids, Rituxan (anti-CD20), etc (in evolution).
4. **ARDS:** Garden variety. Diffuse pulmonary infiltrates and significant hypoxemia. DAD to the pathologist. Concerning clinical overlap with humoral rejection. Outcomes probably worse in lung transplant recipients but no good published studies.
5. **Post Transplantation Lymphoproliferative Disease (PTLD):** Related to primary EBV infection. Present with lung nodule(s). Dx: EBV viral load by PCR but pathology (FNA, core or VATS biopsy) is the gold standard. Studies do not demonstrate that prognosis is dependent on Frizzera categorization or tumor clonality. Vast majority respond to reduced immunosuppression (+/- antiviral therapy). Chemotherapy is not indicated for early PTLD. EBV viral load starts dropping before tumor regression by scans.

Medical Management: Beyond the First Year (generally listed from most to least common)

1. Infections:

(A) Usually bacterial from community acquired organisms.

(B) CMV, fungal and other infections are less common as time goes by.

(C) Community viruses more common: Dx: clinical usually although viral cultures/probes may help. Pathology is nonspecific (DAD).

2. **Acute Vascular Allograft Rejection:** Less common as time goes by but not unheard of.

3. **Humoral Allograft Rejection:** Less common as well.

4. **Chronic Rejection (CR: aka Obliterative Bronchiolitis or Bronchiolitis Obliterans Syndrome):**

Affects >50% of all lung transplant survivors and is the leading cause of death. Mean time to presentation is 1.5-2 yrs. CR is usually a clinical diagnosis of exclusion after all disorders that decrease lung function have been excluded. Patients often suffer larger declines in FEF25-75 than FEV1 early on. Lung function decline is variable but usually much faster than most described lung diseases (COPD, asthma, IPF). CXR is usually normal since it is an airway disorder. The BOS severity scoring system is based on FEV1 decline relative to highest post-transplant FEV1.

Dx is confirmed with transbronchial biopsy (TbBx) pathology in a minority of patients (15%).

While the ISHLT has promulgated a “B” scoring system for airway inflammation, it does not correlate well with CR diagnosed by the BOS scoring system and thus the FEV1 trumps the “B” scores when it comes to CR. Thus, when the biopsy receives a “B” score, the clinician needs to decide what is causing the airway inflammation (I/R injury, infection, CR, other). Rx: augmented immunosuppression, azithromycin, photophoresis, retransplantation, other. There is no convincing evidence that any of these therapies fundamentally alters the disease course, which is highly variable in natural history. The 5 yr survival of CR/BOS is ~30%. Less than 10% of lung transplant patients achieve operational tolerance (do not develop BOS) possibly due to reduced innate-adaptive cross talk, reduced allograft signaling for immune cell recruitment or enhanced regulatory T cell activities.

5. **ARDS:** as above.

6. **Hyperinflation of native COPD lung:** Clinically the patient presents with slowly declining lung function confounding the diagnosis of BOS. Nothing for a pathologist to be concerned with per se but it can ramify into the discussion of histological diagnosis of OB/BO.

7. **Post Transplantation Lymphoproliferative Disease (PTLD):** Related to reactivation EBV infection now with presentation described above. Considerably more difficult to treat. Reduced immunosuppression is the first step unless tumor is of the Burkitt’s type. Chemotherapy is indicated for nonresponsive late PTLD. Mortality is in the range of 50%.

8. **Bronchogenic Cancer:** presents as nodules or rapidly growing masses in the native or donor lung. It is in the Ddx for PTLD, fungal infection or “round” pneumonia. Pathology is diagnostic.

8. **Recurrence of Original Disease:** Best described for sarcoidosis and most reports suggest histologic recurrence without overt disease progression.

9. **Sirolimus-induced Lung Disease:** Incidence unknown but felt to be uncommon. However it may be more frequent in the late (beyond 1 yr) use of sirolimus (recent study in Heart Transplant recipients reported a 24% incidence). Probably easier to diagnose in heart, kidney, liver and other non-lung Tx patients. Broad range of presentations from insidious to fulminant with dry cough and dyspnea usually within the first 6 months after drug initiation. Diffuse interstitial or alveolar infiltrates can mimic CHF, ARDS, other ILDs, or infection. Diagnosis: BOOP, NSIP, or non-necrotizing granuloma on lung biopsy. The pathology of BOOP may share similarities with BO (CR) especially on TbBx where the tissue sample size is small. Clinical Dx mandates a rule out of other causes and disease regression (usually) when the drug is withheld or high dose steroids are given (reminder: most patients are on low dose steroids already). Also note several cases of diffuse alveolar hemorrhage (DAH) have also been reported (pathology: vasculitis).

10. **Aspiration:** Micro-aspiration is common but it is not clear if this causes pulmonary infiltrates or aspiration pathology. The main concern is that this process may augment BOS.