Clinical aspects and application of laboratory tests in viral opportunistic infections

(Adenovirus, Polyomavirus, Influenza virus)

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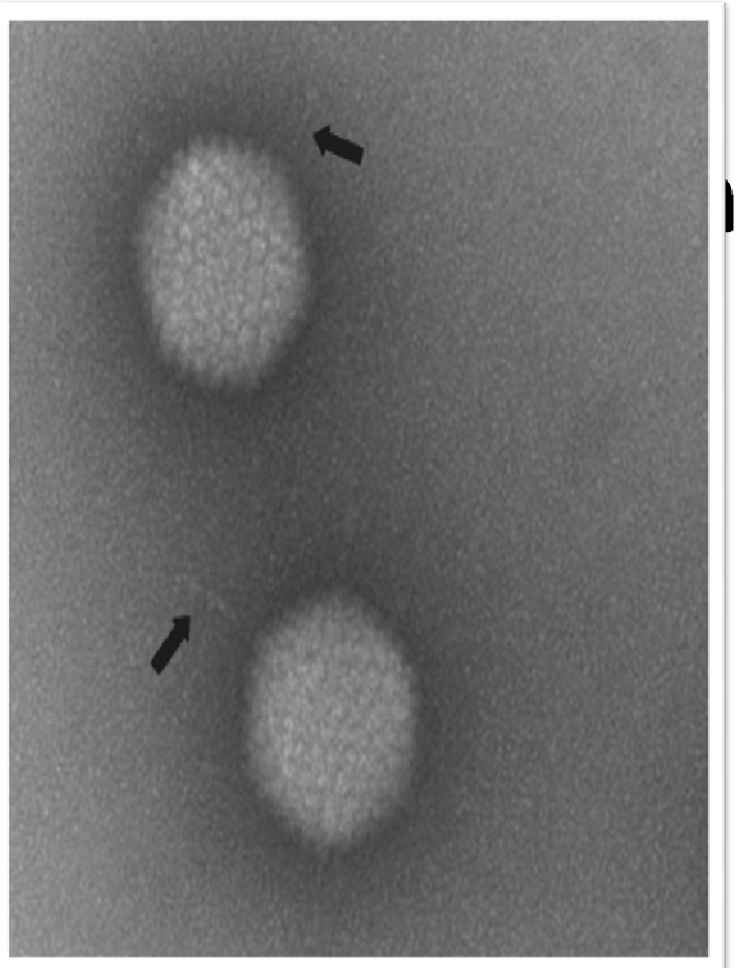


noviruses

Adenoviruses

- 20 y after the discovery of adenoviruses, more than 30 different adenovirus types were identified and were shown to cause several clinical syndromes, including :
  - **upper and lower respiratory tract infections**
  - **keratoconjunctivitis**
  - **infantile gastroenteritis**

Adenoviruses

- DNA viruses
- very common
- causing 5-10% of all febrile illnesses in infants and young children
- mild and self-limiting in immunocompetent patients
- outbreaks of severe respiratory disease with significant morbidity and occasional deaths in **neonates** and **military** and, more recently, in civilian populations
- serious opportunistic pathogen in immunocompromised patients (HSCT and SOT)

Classification of Adenoviruses

GROUP	HEMAGGLUTINATION GROUPS	TYPES	COMMON SITES OF INFECTION
A	IV (little or no agglutination)	12, 18, 31	GI tract, respiratory tract
B	I (complete agglutination of monkey erythrocytes)	3, 7, 11, 14, 16, 21, 34, 35, 50, 55	Respiratory tract, genitourinary tract
C	III (partial agglutination of rat erythrocytes)	1, 2, 5, 6, 57	Respiratory tract, liver
D	II (complete agglutination of rat erythrocytes)	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51, 53, 54, 56, 58-60	Eye, GI tract
E	III	4	Respiratory tract
F	III	40, 41	GI tract
G	III	52	GI tract

Adenoviruses

- portal of viral entry often appears to determine the primary site of disease:
  - spread of ARD by respiratory droplets
  - Spread of infantile diarrhea by fecal-oral transmission
- other organ-limited diseases, such as hemorrhagic cystitis, result from a viremic phase of infection
- Tissue tropism :
  - group C, E, and some B viruses typically infect the respiratory tract
  - group D viruses can cause ocular and GI infections
  - group A, F, and G viruses target the GI tract

EPIDEMIOLOGY

- Ubiquitous
- Most individuals have serologic evidence of prior adenovirus infection by age 10 y
- majority of adenovirus infections remain undocumented
- 50% of all adenovirus infections result in subclinical disease
- most symptomatic infections are mild and self-resolving

Adenoviruses

- Sporadic outbreaks of pediatric infections :
  - daycare centers
  - summer camps
  - public swimming pools
- Severe or fatal adenovirus infections in immunocompetent adults
  - Rare
  - in 2006 and 2007, several clusters of severe ARD were caused by adenovirus type 14 that affected military personnel, infants, and immunocompetent adults

Transmission

- respiratory droplets
- fecal-oral
- contact with contaminated fomites
- Nosocomially (in outbreaks of keratoconjunctivitis and respiratory disease on hospital wards)

CLINICAL SYNDROMES

CLINICAL DISEASE	POPULATIONS AT RISK	CAUSAL ADENOVIRUS TYPES
Pharyngitis	Infants, children	1-7
Pharyngoconjunctival fever	Children	3, 7
Pertussis-like syndrome	Children	5
Pneumonia	Infants, children	1-3, 21, 56
	Military recruits	4, 7, 14
Acute respiratory disease	Military recruits	3, 4, 7, 14, 21, 55
Conjunctivitis	Children	1-4, 7
Epidemic keratoconjunctivitis	Adults, children	8, 11, 19, 37, 53, 54
Gastroenteritis	Infants	31, 40, 41
	Children	2, 3, 5
Intussusception	Children	1, 2, 4, 5
Hemorrhagic cystitis	Children	7, 11, 21
	HSCT recipients, renal transplant recipients	34, 35
Meningoencephalitis	Children, immunocompromised hosts	2, 6, 7, 12, 32
Hepatitis	Pediatric liver transplant recipients	1-3, 5, 7
Nephritis	Renal transplant recipients	11, 34, 35
Myocarditis	Children	7, 21
Urethritis	Adults	2, 19, 37
Disseminated disease	Neonates, immunocompromised hosts	1, 2, 5, 11, 31, 34, 35, 40

Respiratory Tract Disease:

- In children, cause 5% of URTI (mild pharyngitis or tracheitis accompanied by coryza) and 10% of pneumonias
- fever, malaise, headache, myalgia, and abdominal pain
- Exudative tonsillitis and cervical adenopathy may be present and can be clinically indistinguishable from group A streptococcal infection
- otitis media (In children younger than 1 year)
- pertussis-like syndrome
- pneumonia in children

Respiratory Tract Disease:

- outbreaks of ARD have been documented in military recruits:
  - fever, sore throat, cough, hoarseness, and rhinorrhea and may progress to involve the lower respiratory tract
  - Symptoms usually last 3-5 d
  - Ph E: pharyngitis, râles, and rhonchi
  - CXR: bilateral patchy ground-glass opacities
  - Rare extrapulmonary complications: meningoencephalitis, hepatitis, myocarditis, nephritis, neutropenia, and DIC

Ocular Disease:

- Pharyngoconjunctival fever:
  - a common syndrome
  - benign follicular conjunctivitis, fever, pharyngitis, and cervical adenitis
  - commonly caused by adenovirus types 3 and 7
  - Palpebral and bulbar conjunctivitis may be the sole finding and is typically bilateral
  - a common sporadic illness in children
  - associated with outbreaks in children's summer camps, swimming pools, and lakes
  - usually mild and self-limited

Ocular Disease:

- **EKC**
  - more serious illness
  - unilateral or bilateral follicular conjunctivitis
  - corneal subepithelial infiltrates
  - painful and can cause blurry vision
  - Prominent preauricular lymphadenopathy
  - incubation period : 8 to 10 d
  - virus can be isolated for up to 9 d after the onset of symptoms
  - usually self-limited
  - can take up to 1 month to resolve
  - Corneal opacities may persist for several months to years after infection
  - highly contagious
  - outbreaks in schools, military bases, and hospital wards
  - Transmission by instruments, eye drops, and skin has been documented in ophthalmic practices

Gastrointestinal Tract Disease

- Acute infantile gastroenteritis :
  - a watery diarrhea for 8-12d
  - fever and vomiting
  - 2-5% of acute diarrheal illnesses in young children→ adenoviruses 40 and 41

- mesenteric adenitis:
  - Adenoviruses 1, 2, 5, 6
  - mimic appendicitis
  - Can cause intussusception

Genitourinary Tract Disease

- **Acute hemorrhagic cystitis:**
  - benign, self-limited illness
  - gross hematuria for 3d, without fever or hemodynamic instability
  - Microscopic hematuria and dysuria may persist for several more days
  - Boys > girls
  - Adenovirus types 11, 21 (the most commonly isolated)
  - Specially in RT and SCT

CNS Disease

- sporadic cases of meningitis and meningoencephalitis
- primary or as a complication of systemic or respiratory infection
- Rarely adenovirus has been cultured only from CSF in immunocompetent patients and patients undergoing chemotherapy for lymphoma

Other Clinical Syndromes

- Myocarditis (myocardial tissue PCR +)
- myositis associated with rhabdomyolysis,arthritis,and pancreatitis (rare)
- Disseminated adenoviral disease (in pediatric and HSCT)

INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

- from asymptomatic shedding to disseminated and life-threatening disease
- ↑ incidence in HSCT and SOT because of:
  - improvements in diagnostic methods
  - more aggressive conditioning regimens
  - institution of surveillance for adenoviral infection by PCR at some centers
- more likely to have coinfection with more than one adenovirus type

HSCT

- mortality rates : 6% to 70%
- Risk factors:
  - younger age
  - unrelated donor
  - graft-versus-host disease
  - cord transplants
  - aggressive immunosuppression
  - total body irradiation
  - Low T-lymphocyte counts after transplantation

HSCT

- usually detected within the first 100 days post-transplant
- ↑ risk for serious clinical disease:
  - presence of adenovirus DNA in blood
  - greater degree of immunosuppression
  - Lymphocytopenia
  - rising viral load
- In pediatric HSCT population, the most common adenoviral disease is diarrhea or GE

HSCT

- Surveillance blood PCR :
  - a common practice in some pediatric and adult HSCT centers
  - can be detected in blood 2 or 3 weeks before the development of clinical symptoms
  - ↑VL is associated with ↑ mortality once clinical disease is established

SOT

- the transplanted organ is typically the primary site of disease
- primary or reactivation of latent virus in the transplanted organ
- more common in children and in patients with D+/R- adenovirus status
- ↑Severe disease:
  - pediatric transplant population, particularly liver and lung recipients
  - receive antilymphocyte antibodies

SOT

- Adenovirus hepatitis :
  - In pediatric liver transplant recipients
  - frequently led to graft loss and death
  - mortality rates up to 53%
  - Most commonly : type 5
- Lung transplant recipients →adenovirus pneumonia in the early post-transplant period → graft failure, death, or BO
- Renal transplant recipients → acute hemorrhagic cystitis +/- tubulointerstitial nephritis

DIAGNOSIS

- **diagnosis is useful :**
  - in the setting of outbreaks
  - for individuals who are immunosuppressed
  - seriously ill patients
- **Methods:**
  - viral culture
  - antigen-specific assays
  - Serologies
  - PCR
  - Histopathology

DIAGNOSIS

- **viral culture**

  Viruses may be recovered from:

  - nasopharyngeal swabs or aspirates
  - throat swabs
  - conjunctival swabs or scrapings
  - stool or rectal swabs
  - Urine, CSF, tissue

- **Viral excretion is detectable in:**

  - first 1-3 d in patients with pharyngitis
  - 3-5 d in pharyngoconjunctival fever
  - up to 2w in keratoconjunctivitis

DIAGNOSIS

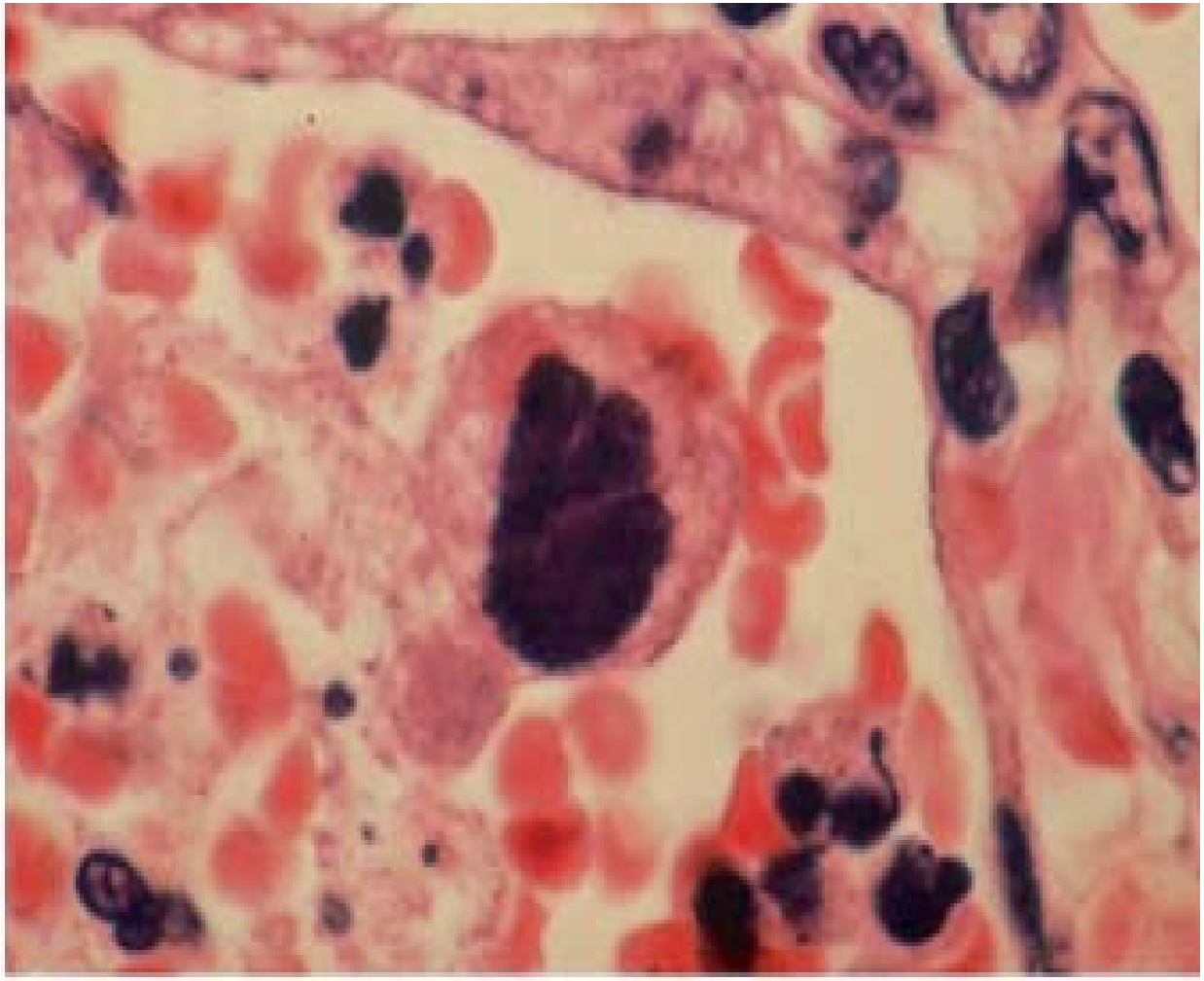
- Ag-specific assays
  - **IFA:** useful for respiratory samples and tissue
  - **ELISA**: choice for adenoviruses 40 ,41 in stool
- **Serologies**
  - detecting a fourfold or greater rise in adenovirus-specific Ab titers in paired acute and convalescent sera
- **PCR:**
  - High specificity in asymptomatic, immunocompetent adults (96-100%) in studies of urine, throat swabs, and peripheral blood

DIAGNOSIS

- **Histopathologic findings in the lung:**
  - diffuse interstitial pneumonitis, necrotizing bronchitis, bronchiolitis, and pneumonia with mononuclear cell infiltration and hyaline membrane formation
  - Early postinfection, infected cells may display small eosinophilic inclusions
  - During late infection, basophilic intranuclear inclusions surrounded by a thin, clear halo emerge and eventually enlarge to obscure the nuclear membrane⇨ **"smudge cells,"** (characteristic of adenovirus infections)
  - In contrast to CMV, there are no intracytoplasmic inclusions or multinucleated giant cells

DIAGNOSIS

- “smudge cell” in a Lung biopsy specimen



Therapy

- immune reconstitution (with improved absolute lymphocyte counts and CD4 T-cell counts)
- Cidofovir (invitro and animal models)
- oral liposomal formulation of cidofovir (CMX001)
- Ribavirin
- Vidarabine and ganciclovir
- Immunotherapy by adoptive T-cell therapy
- IVIG

Polyomaviruses

Polyomaviruses

- double-stranded DNA
- JC virus (JCV) and BK virus (BKV)
- Ubiquitous
- do not cause disease in in immunocompetent individuals
- 90% and 86% of the general adults are seropositive for BKV and JCV, respectively

VIROLOGY

- nine new members of the Polyomavirusgenus
  - the site of discovery:
    - WUPyV (Washington University)
    - KIPyV (Karolinska Institute)
  - their geographic origins:
    - MWPyV (Malawi)
    - STLPyV (St Louis)
  - the diseases that they cause:
    - MCPyV (Merkel cell carcinoma)
    - TSPyV (trichodysplasia spinulosa)
  - Their order of discovery: human polyomaviruses 6, 7, and 9

Epidemiology

- Both JCV and BKV have worldwide distribution
- **JC viruria:** occurs independent of the host's immune status
- **JC viremia :** usually only in immunosuppressed patient
  - HIV+ and PML- : 20-40%
  - HIV+ and PML+: 60-80%

Epidemiology

- **BK viruria:**
  - asymptomatic immunocompetent individuals : 0-20%
  - immunosuppression : 10-60% (correlates with the degrees of immunosuppression)
  - in the urine of patients who are HIV+, ↑ BK VL with ↓CD4+ T-cell counts
- **BK Viremia:**
  - usually not detected in either immunocompetence or immuno suppressed
  - detection of BKV DNA in the plasma of renal transplant patients is an indication of development of BKV-induced nephropathy

Polyomaviruses–ASSOCIATED SYNDROMES

- **PML**
  - occur in 0.07% of individuals with hematologic malignancies, or rarely in patients with solid-organ cancer or organ transplants
  - 0.6 to 1.3/1000 HIV-positive person-years
  - Underlying disease in patients with PML:
    - AIDS: 82%
    - hematologic malignancies: 8.4%
    - SOT: 2.8%
    - chronic inflammatory diseases : 0.95%
    - no defined risk factor for PML : 6%

Polyomaviruses–ASSOCIATED SYNDROMES

- a new emerging group of patients with PML:

  patients treated with immunomodulatory medications for malignant diseases or autoimmune diseases, such as

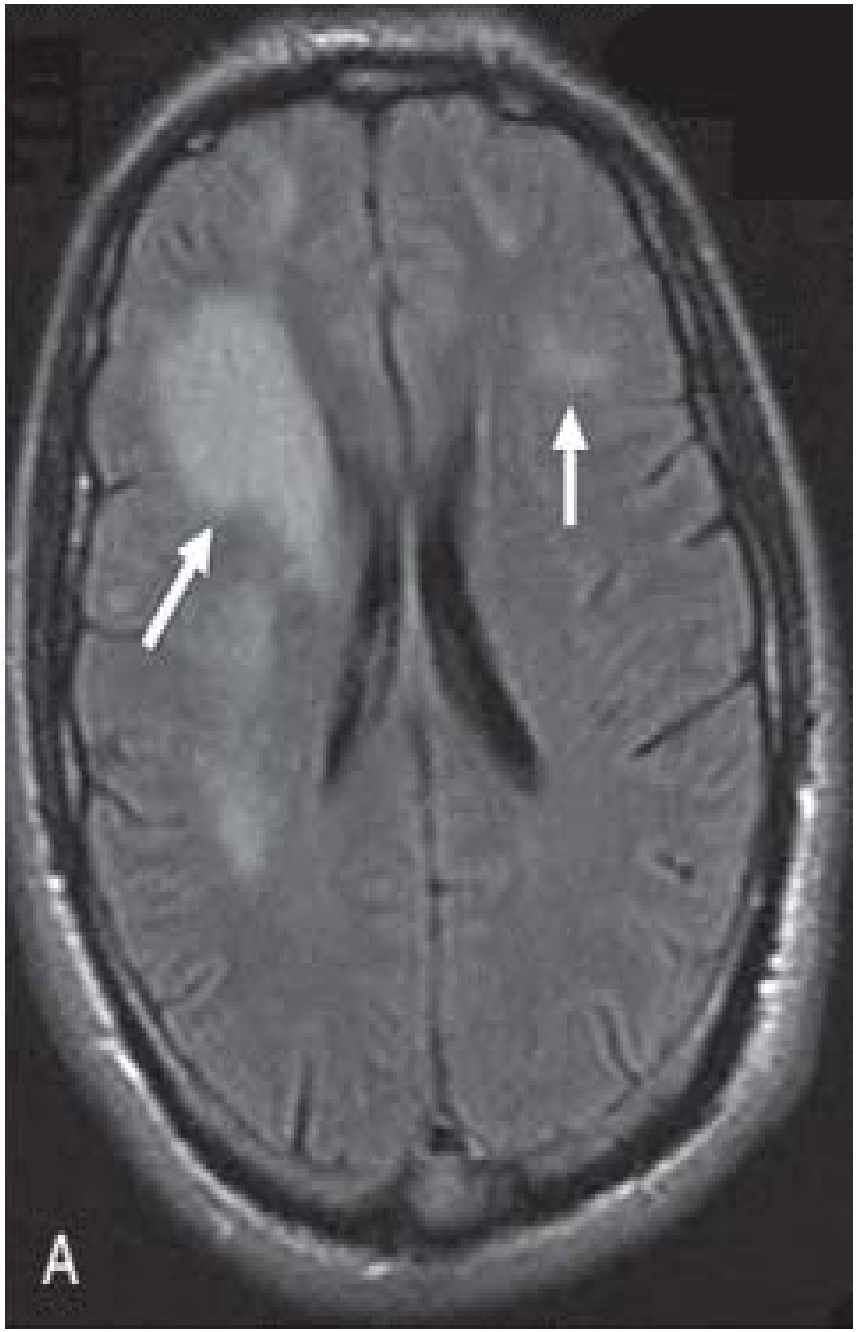
  - natalizumab for MS or Crohn's disease
  - rituximab for lymphoma or lupus
  - efalizumab or fumaric acid for psoriasis

PML Clinical Manifestations

1. **CLASSIC PML:**
   - Subacute onset
   - MRI:
     - Asymmetrical, well-demarcated, non enhancing subcortical white matter lesions
     - Hyperintense on T2-weighted and FLAIR images
     - Hypointense on T1-weighted images
   - Neurologic symptoms:
     - Based on location → coordination difficulties, gait imbalance, cognitive dysfunction, visual problems, and limb paresis
     - Seizures (18%)
   - Histology:
     - Demyelinating lesions often at gray-white matter junction
     - JCV detected in enlarged oligodendrocytes; bizarre astrocytes
     - Presence of CD8+ T cells near JCV-infected cells

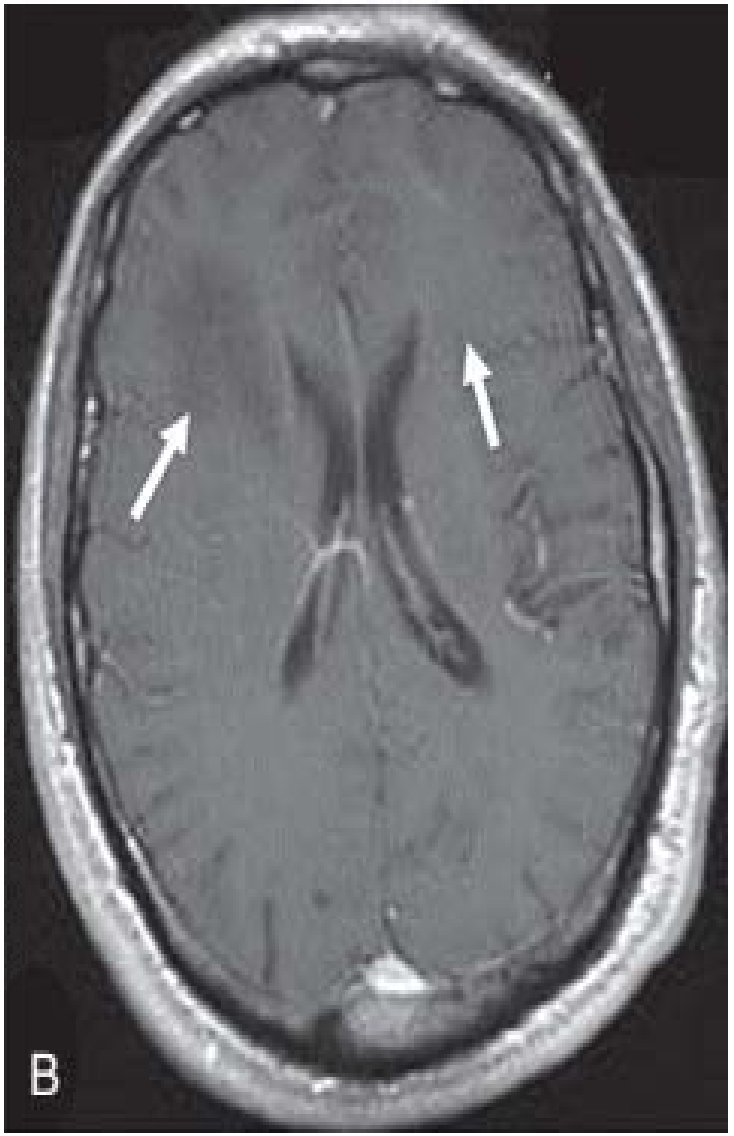
PM

- FLAIR image shows lesions in right and left frontal lobes (arrows)



PML

- No enhancement is evident after gadolinium injection on a T1-weighted image (arrows)

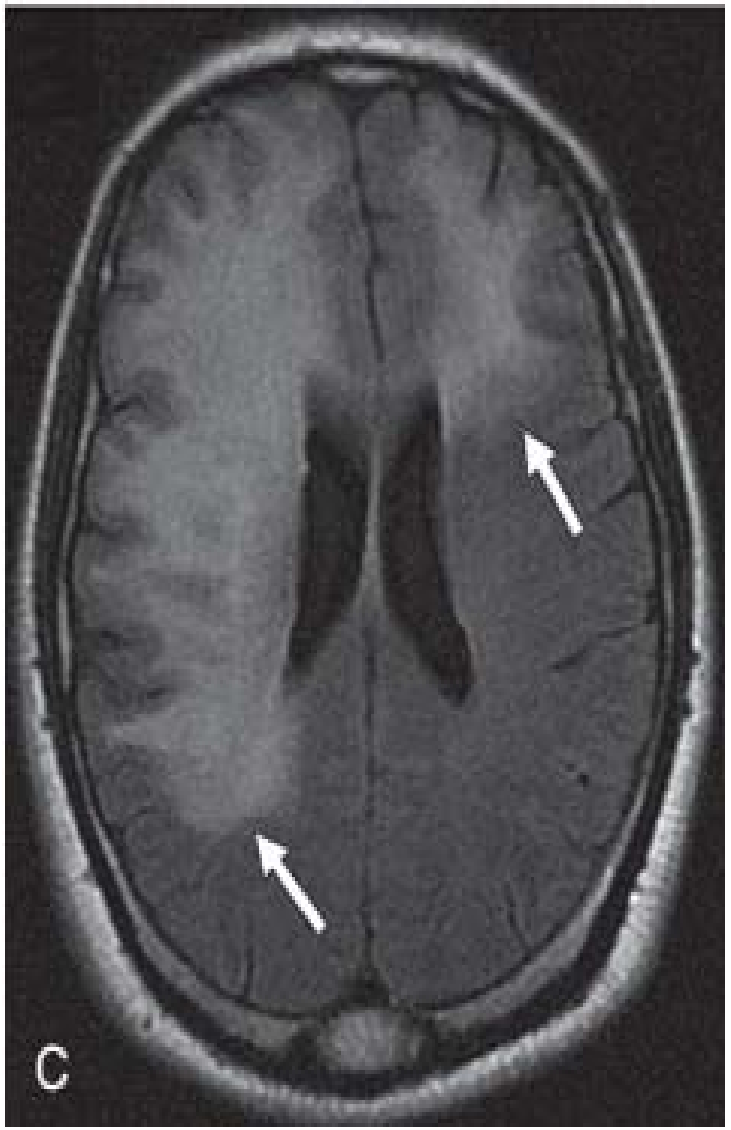


Clinical Manifestations

2. PML-IRIS:
   - Onset: Immune recovery
   - MRI: Contrast-enhancing lesions and possible mass effect
   - Neurologic symptoms: Based on location and inflammation
   - Histology: Demyelination similar to classic PML but with marked inflammatory infiltrates

PML-IRIS

- FLAIR image after ART shows PML-IRIS displaying progression of lesions in both hemispheres (arrows)



Clinical Manifestations

3. JC Virus Granule Cell Neuronopathy
   - Onset: Chronic
   - MRI: Cerebellar atrophy
   - Neurologic symptoms: Cerebellar syndrome (cerebellar atrophy, gait ataxia, and incoordination)
   - Histology: Focal areas of cell loss in granule cell layer of cerebellum; JCV detected in enlarged granule cell neurons

4. JC Virus Encephalopathy:
   - Onset: Subacute
   - MRI: Cortical lesions
   - Neurologic symptoms: Encephalopathy (global cognitive decline and aphasia)
   - Histology: Focal areas of cell loss in hemispheric cortex; JCV detected in enlarged pyramidal neurons

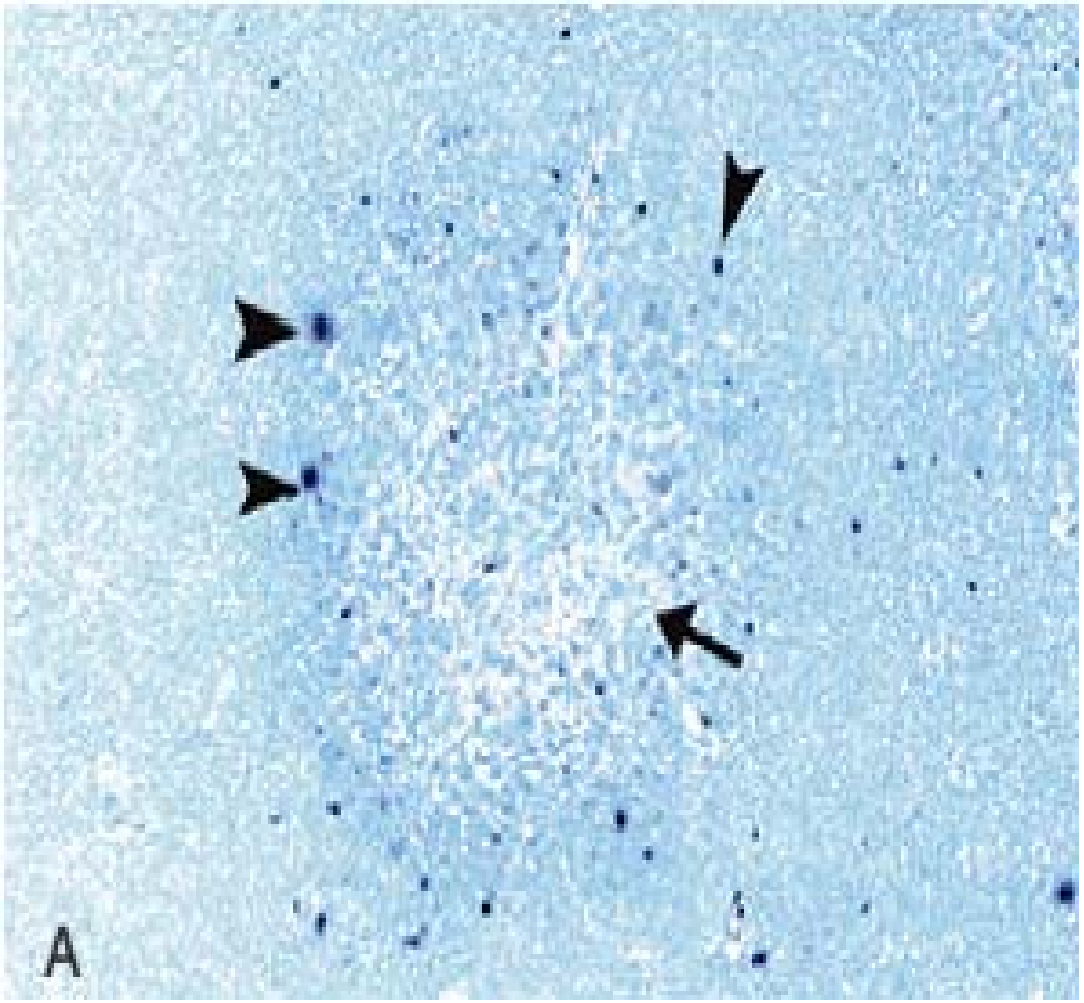
Diagnosis

- Histopathology: demonstration of JCV infection in the brain

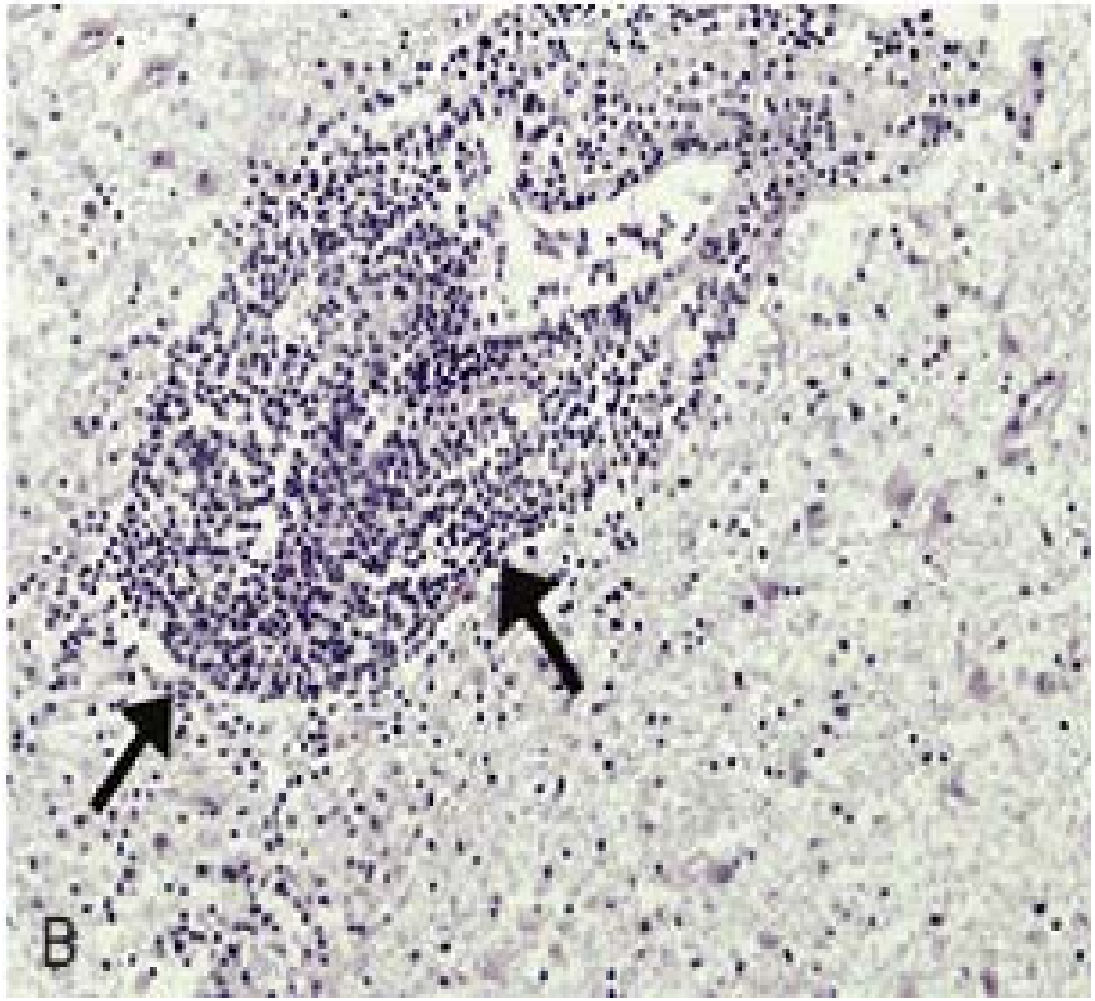
Brain Biopsy

- gold standard for the diagnosis
- Sensitivity: 64-96%
- specificity : 100%
- Histology: demyelinated areas, with reactive gliosis and enlarged and bizarre astrocytes, and macrophages that contain phagocytosed myelin and cellular debris

- Classic PML,with demyelinating lesion in white matter (arrow) surrounded by multiple JCV infected glial cells (dark blue, arrowheads)



- PML-IRIS, with marked lymphocytic perivascular infiltrates (arrows)



CSF-PCR

- nonspecific CSF profile:
  - mild pleocytosis
  - slightly elevated protein
  - normal glucose levels
- The detection of JCV with PCR assay of CSF:
  - sensitivity of 72% to 92% (post-ART era : 58%)
  - specificity of 92% to 100%

NEPHROPATHY AND OTHER BK VIRUS–ASSOCIATED DISEASES

- Epidemiology:
  - BKV-induced nephropathy
    - prevalence in KT: 1-10%
    - peak : 24 weeks after KT
    - potent immunosuppressive drugs(tacrolimus & mycophenolate) → ↑ prevalence
  - BKV-induced ureteral stenosis :
    - prevalence in KT: 3%
  - hemorrhagic cystitis:
    - the most prevalent BKV-associated complication
    - occurs in 10% to 25% of BMT

BKV Clinical Manifestations

1. Nephropathy:
   - Asymptomatic hematuria
   - hemorrhagic and nonhemorrhagic cystitis
   - ureteral stenosis
   - interstitial nephritis (in HIV or renal transplantation)
   - BK viruria and viremia usually precede nephropathy
   - Clinical manifestations of BKV nephropathy :
     - slowly increasing serum creatinine levels without symptom
     - Hematuria
     - fever

BKV Clinical Manifestations

2. Ureteral Stenosis
   - urinary obstruction
   - ↑serum Cr
   - No pain or discomfort because the transplanted kidney is not innervated

BKV Clinical Manifestations

3. Hemorrhagic Cystitis
   - 50% of HSCT patients have BK viruria develop within 2 m of transplant, usually after engraftment
   - Diagnosis :
     - postengraftment BMT patients with hematuria, dysuria, urgency, frequency, or suprapubic pain
     - severe bleeding and clot formation
   - complications : urinary tract obstruction and renal failure

BKV Infections Outside the Renal System

- Encephalitis
- pneumonia and pneumonitis

Diagnosis

1. Urine PCR
   - Detection of BKV in urine often precedes viral detection in the blood
   - sustained viruria:
     - detection in two or more consecutive urine samples
     - 100% sensitive and 94% specific for BK viremia
   - Urine cytology containing decoy cell (enlarged nucleus with a single large basophilic intranuclear inclusion) : not specific to BKV infection(JCV and adenovirus)
   - Urine BKV DNA in patients with hemorrhagic cystitis :
     - Sensitive
     - nonspecific (asymptomatic BK viruria is common)

Diagnosis

2. **Renal Biopsy:**
   - often use in the diagnosis of nephropathy
   - false-negative rate up to 30% because of the focal nature of the disease

3. **Plasma PCR:**
   - more useful in ruling out BKV nephropathy than in diagnosing it
   - For nephropathy: NPV: 100% and PPV: 50%
   - usually not detected in the blood of patients with hemorrhagic cystitis or ureteral stenosis

enza Virus

Classification

- belong to the family Orthomyxoviridae
- classified into three distinct types:
  - influenza A virus
  - influenza B virus
  - influenza C virus
- Influenza A and B : outbreaks and epidemics
- influenza A : more severe and widespread disease
- Influenza C : sporadic upper respiratory tract illness

	INFLUENZA A	INFLUENZA B	INFLUENZA C
Genetics	8 gene segments	8 gene segments	7 gene segments
Structure	10 viral proteins M2 unique	11 viral proteins NB unique	9 viral proteins HEF unique
Natural host range	Humans, swine, equine, birds, marine mammals*	Humans only	Humans and swine
Epidemiology	Antigenic shift and drift	Antigenic drift only; two main lineages cocirculate	Antigenic drift only; multiple variants
Clinical manifestations	May cause large pandemics with significant mortality in young persons	Severe disease generally confined to older adults or persons at high risk; pandemics not seen	Mild disease without seasonality

CLINICAL MANIFESTATIONS

Uncomplicated Influenza

- abrupt onset of symptoms after an incubation period of 1 to 2 days
  - Feverishness
  - chilliness or frank shaking chills
  - Headaches
  - Myalgia
  - Malaise
  - Anorexia
  - arthralgia
  - tearing and burning

Complications of Influenza

Pulmonary Complications

	PRIMARY VIRAL PNEUMONIA	SECONDARY BACTERIAL PNEUMONIA	MIXED VIRAL AND BACTERIAL PNEUMONIA	LOCALIZED VIRAL PNEUMONIA
Setting	Cardiovascular disease; pregnancy; young adult (pH1N1)	Adults and children	Any associated with A or B	?Normal
Clinical history	Relentless progression from classic 3-day influenza	Improvement, then worsening after 3-day influenza	Features of both primary and secondary pneumonia	Continuation of classic 3-day syndrome
Physical examination	Bilateral findings, no consolidation	Consolidation	Consolidation	Area of rales
Sputum bacteriology	Normal flora	*Pneumococcus, Staphylococcus, Haemophilus influenzae*	*Pneumococcus, Staphylococcus H. influenzae*	Normal flora
Chest radiography	Bilateral findings	Consolidation	Consolidation	Segmental infiltrate
Detection of influenza virus	Yes	Not always	Yes	Yes
Response to antibiotics	No	Yes	Often	No
Mortality	High	Variable	Variable	Very low

Complications of Influenza

- **Nonpulmonary Complications:**
  - Myositis
  - Cardiac Complications
    - myocarditis
    - Pericarditis
  - Toxic Shock Syndrome
  - CNS Complications
    - Guillain-Barré syndrome
    - transverse myelitis
    - encephalitis

Complications of Influenza

- **Nonpulmonary Complications:**
  - Reye Syndrome:
    - Range from lethargy to delirium, obtundation, seizures, and respiratory arrest
    - LP ⇨ Pro:NL Cell: Nl
    - in children who have been given aspirin to treat febrile illnesses due to influenza and other viruses

DIAGNOSIS

1. Clinical Diagnosis
2. Rapid Influenza Diagnostic Tests
3. Molecular Diagnostic Tests
4. Serology
5. Virus Isolation

Rapid Influenza Diagnostic Tests

- immunologic detection of viral Ag in respiratory secretions
- a sample of respiratory secretions is treated with a mucolytic agent and then tested, either on a filter paper, in an optical device, or with a dipstick in which reaction with specific antibody results in a color change
- none of the current tests distinguishes between influenza A/H1N1 and A/H3N2 viruses
- simple and rapid
- Sensitivity: 40-80%

Rapid Influenza Diagnostic Tests

- ↑ sensitivity:
  - young children > adults and older adult
  - early in the course of illness
  - nasopharyngeal swabs and aspirates > throat swabs or gargles

Molecular Diagnostic Tests

- PCR is more sensitive than cell culture
- increasingly becoming the primary diagnostic testing modality in hospital-based diagnostic laboratories
- ↑ sensitivity in nasopharyngeal swabs sample

Serology

- Eg: complement fixation and hemagglutination inhibition
- Because most individuals have been previously infected with influenza viruses, a single serum sample is generally not adequate, and paired serum specimens, consisting of an acute serum specimen and a convalescent serum specimen obtained 10 to 20 days later, should be submitted for testing

Virus Isolation

- from nasal swab specimens, throat swab specimens, nasal washes, or combined nose and throat swab specimens
- Samples should be placed into containers of viral transport medium and transported to the laboratory as soon as possible
- virus is detected by cytopathic effect or hemadsorption in monkey kidney
- > 90% of positive cultures can be detected within 3d of inoculation and the remainder by 5 -7 d

Role of Viral Diagnosis in Clinical Decision Making

- Most typical cases → No need for specific viral confirmation
- Use diagnostic testing for:
  - decisions regarding the use of antiviral agents
  - the need for antibacterial drugs
  - considerations for infection control
- During the influenza season, certain groups should be considered for diagnostic testing
- Outpatients with higher risk for influenza complications should be considered for testing if they are presenting within 5d of symptom onset

Role of Viral Diagnosis in Clinical Decision Making

- Considered outpatients for testing even beyond 5d:
  1. Immunocompromised
  2. Elderly
  3. Infants
  4. Hospitalized patients with febrile illness during influenza epidemics

- Most positive rapid tests outside the influenza season: false positive
- typical influenza symptoms during epidemic with a negative rapid test → receive antiviral therapy

THANKS FOR YOUR ATTENTION!