Viral Molecular Diagnosis in Transplantation

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Viral infection in Transplantation

- Perturbations of immunity can influence the pathogenesis of viral infections
  - Prominent among these: many herpesvirus infections in immunocompromised hosts.
    - Herpes simplex, CMV, EBV, VZV, HHV-6 human herpesvirus 6, HHV- 7 human herpesvirus 7
  - Polyomavirus type BK and JC virus.
  - Adenovirus, Influenza, hMpv, RSV, Parainfluenza, Rhinovirus.

Which techniques should be used in Clinical Diagnosis of Viral infection

Criteria;

- Fast, Sensitive, Specific, Simple to conduct

Viral detection techniques

- Electron microscopy
  - Insensitive & laborious
- Cell culture
  - Time consuming, relative insensitive, large part of clinically relevant viruses can not be grown
- Immunoassays (Serology)
  - Indirect & depends on host immune system
- Direct detection (Antigen & Nucleic Acid)
  - NAT; universally applicable, revolutionary technology

Human Cytomegalovirus

HCMV

CMV continues to be an important omplication after transplantation

inical complications of HCMV

- Gastrointestinal disease;
  - can escape blood-based surveillance by PCR and the pp65 antigenemia assay in approximately 25% of patients
- HCMV pneumonia; most serious complication
- Rare manifestations include retinitis and encephalitis
- invasive bacterial and fungal disease as well as GVHD

- **Diagnosis of HCMV infection and disease**
  - Histopathology
    - confirms the presence of tissue-invasive CMV disease
    - invasive procedure but recommended
      - another concomitant pathology (e.g. graft rejection) or copathogens or negative HCMV testing in the blood
      - repeated histopathology not clinically necessary
  - Culture; specific but modest sensitivity and slow turn-around time
  - Serology; limited utility for diagnosis
    - delayed or impaired ability to mount an antibody response
  - antigenemia pp65
  - molecular assays
    - Q-NAT
    - Late mRNA pp67

HCMV Diagnosis

ntigenemia assay pp65

Semiquantitative assay in HCMV-infected peripheral blood leukocytes

Higher sensitivity than culture & and is comparable to NAT (PCR)

Useful to guide

- preemptive therapy, for rapid and sensitive diagnosis of HCMV disease,
- treatment responses

Main disadvantage

- Subjective method
- need to process the clinical sample within few hours
- limited utility in leukopenic patients

Molecular tests

- Detection of HCMV DNA (QNAT) or Late mRNA (pp67) are the preferred methods for the diagnosis of HCMV
  - HCMV RNA is indicative of active HCMV replication
  - In contrast, detection of HCMV DNA may or may not reflect HCMV replication
    - a highly sensitive NAT may amplify latent viral DNA
  - Hence, QNAT assays can differentiate
    - Active viral replication (typically associated with high viral load) from latent virus (low-level HCMV DNAemia if using highly sensitive tests)
    - Higher HCMV load values are generally associated with tissue-invasive disease, while lower values are seen with asymptomatic HCMV infection, and intermediate-range viral loads are seen with HCMV syndrome
      - however, there is wide overlap between these categories
  - **late mRNA-based anti-HCMV therapy show comparable safety and efficacy with PCR-based therapy in patients after Transplantation**

HCMV Diagnosis

no consensus on how to use molecular methods to conclusively diagnose HCMV pneumonia and gastrointestinal disease

PCR is therefore not accepted as definitive proof of HCMV pneumonia or gastrointestinal disease

- no data on what level of HCMV DNA in BAL fluid or tissue correlates best with HCMV disease
  - negative predictive value of PCR is high, so it can be used to rule out disease

There are occasional patients with tissue-invasive disease (especially late-onset gastrointestinal CMV disease and retinitis) with very low to undetectable viral load in the blood

- these cases may be due to CMV disease compartmentalization, or the use of less sensitive QNAT assays.

In HCMV encephalitis, PCR is a useful diagnostic tool

Threshold Quantities; no clear recommendation

PAST, the biggest drawback of HCMV
T was the variability of test results
oss laboratories due to the lack of
ay standardization

- differences in commercial detection reagents, calibration, nucleic acid extraction methods and the selection of primers and probes targeting different genes
- up to a 3 log10 difference in viral load values across different laboratories
- Significant variations in viral load may be due to SAMPLE TYPE
  - WHOLE BLOOD samples is more sensitive and yields higher viral load and earlier time to viral detection compared to PLASMA samples
  - In a study which compared plasma versus whole blood for monitoring of CMV levels during treatment of CMV disease, there was a higher rate of detectable virus at day 21 in the whole blood samples when compared to the plasma samples (70% versus 52%)

- In 2011, the WHO released the fir
  International Reference Standard f
  the quantification of CMV nucleic
  acid
  - CMV QNAT assays should now be calibrated to this standard
  - may ensure uniformity in viral load reporting, thereby facilitating to defi
    viral thresholds for various clinical applications
    - preemptive therapy, disease prognostication, therapeutic monitoring
- Threshold Quantities
  - A study; HCMV load of 250 IU/mL or greate
    plasma / HSCT
  - A study; a viral load of 3,983 IU/mL in whole
    as cut-off for starting pre-emptive therapy in
    patients / SOT

Recommendations for HCMV diagnosis

Viral culture of blood and urine has limited clinical utility for prediction, diagnosis and management of CMV disease in adult patients

Serologic assays to detect HCMV-IgM and IgG antibodies should not be used for the diagnosis of CMV disease

HCMV QNAT or pp65 antigenemia should be used for rapid diagnosis of HCMV disease

HCMV QNAT or pp65 antigenemia should be performed **once weekly** for monitoring the response of CMV disease to antiviral treatment.

HCMV QNAT or pp65 antigenemia should be performed **once weekly** to predict risk of HCMV disease, if preemptive therapy is used for CMV prevention

HCMV QNAT assays should be calibrated based on the WHO International Reference Standard

- Studies should report HCMV load in **IU/mL using QNAT assays** that have been calibrated to the WHO International Reference Standard

Patients suspected to have **tissue-invasive HCMV disease** but with negative QNAT or pp65 antigenemia should have tissue biopsy and **histopathology to confirm** the clinical suspicion of CMV disease

Herpes Simplex virus

Herpes Simplex Virus

Transplant recipients shed virus more frequently, have more frequent and severe clinical manifestations of HSV, and may be slower to respond to therapy.

Most symptomatic HSV disease in adult transplant recipients results from reactivation of previously acquired virus,

- particularly early after transplantation (in the 2nd weeks) and in the setting of antirejection therapy
- Hepatitis & Pneumonitis are the major manifestations
- Keratitis (infection of the cornea) is the most common manifestation of HSV in the eye

HSV in immunocompromised hosts may be atypical, thus, laboratory confirmation may be helpful.

HSV laboratory Diagnosis

- Tissue culture
  - most isolates are identified within 5 days
  - Timing of sampling is important;
    - sampling of genital lesions >5 days old had a yield of less than 35%
- DFA testing; more of mucocutaneous lesions, BAL and other clinical samples, can provide rapid results
- PCR assays
  - up to four fold more sensitive than tissue culture for diagnosing and have replaced viral culture as the preferred diagnostic test
  - diagnostic test of choice for HSV encephalitis
  - Tissue histopathology with immunocytochemistry is helpful and is recommended to confirm a PCR positive diagnosis the samples contaminated from another site (e.g. BAL contaminated from oropharynx)
- Serologic testing is rarely useful for diagnosing acute infections
  - useful to acquire pre-transplant for appropriate post-transplant risk stratification
- Diagnosis of HSV keratitis remains primarily a clinical diagnosis

Adenovirus

ADENOVIRUS

- Not nearly as prevalent as the herpesviruses , adenoviruses have been isolated from transplant recipients, and have contributed to their morbidity and mortality.
- isolation of adenovirus from stool, throat, urine and peripheral blood.
  - The virus often appears in the blood about 3 weeks following the transplant
  - Pneumonia, gasteroenteritis and hepatic abnormalities
  - Adenovirus species A, B, C (78% of all positive cases), D, and F.
    - Ad1, 2, 4, 5, 6, 11,31, 34, and 35 .

Clinical Manifestations

Vary with the sites affected and the type of transplanted organ

- Liver transplant recipients; commonly hepatitis, gastrointestinal tract, respiratory and urinary tract
- lung transplant recipients; acute flu-like illness, diffuse alveolar damage or necrotizing pneumonia and chronic changes such as bronchiolitis obliterans, interstitial fibrosis or bronchiectasis
- Heart transplant recipients; detection of adenoviral genome in myocardial biopsy specimens might be predictive of coronary vasculopathy and graft loss
- In transplants involving the small bowel, enteritis is common and a significant proportion of these patients develop disseminated adenovirus disease
- renal transplant recipients; Hemorrhagic cystitis and graft dysfunction are described more often in adult than pediatric
- Bone marrow transplant recipients; detection of adenovirus at two or more sites has been found to be predictive of invasive disease
    - similar data are not available for organ transplant recipients

Adenovirus
Diagnostic approach

- The site of infection should determine the type of sample to test;
  - STOOL; Gasterointestinal disease
  - Urine; Genitourinary disease
  - Respiratory samples; Pneumonia
- Once disease is documented, Quantitative Viral load test of blood should be consider as a marker for monitoring disease progression and treatment response.
- Various diagnostic techniques have been described:
  - Serology, Antigen detection, Culture, Nucleic acid testing, Pathology

Adenovirus
Diagnostic approach

- Culture; traditional gold standard!
  - Time consuming; several days to weeks
  - Rapid shell vial test; reduced sensitivity.
- PCR is more sensitive
  - More increasingly use; the ease and sesitivity
  - Applied to whole blood as a significant screening method;
    - Documented in pediatric HSCT recipients, not in adults.
- No specific threshold quantity for prediction the disease
  - Higher viral DNA levels($>10^6$ copies/ml) associated with greater risk of death among pediatric transplant recipients.

ADENOVIRUS …

- Adenoviraemia is commonly found among adult SOT recipients and does not predict disease,
  - Should not be used to prospectively screening the disease
    - Possible exception of small bowel transplant recipients.

Dynamic trends in adenovirus load in blood also appear to be useful tool in monitoring response to therapy.

ADENOVIRUS …

- In summary from the various transplant studies
  - PCR or real-time PCR is an effective and sensitive method to detect adenovirus in clinical specimens.
  - Detection of adenovirus in stool and throat swab is usually not associated with adenovirus disease.
  - Detection of adenovirus in the peripheral blood is more often associated with disease, but only very roughly about half the time.
  - The virus often appears in the blood about 3 weeks before the onset of symptoms
    - virus DNA levels in the blood of greater than 10^6 to 10^7 (or more) copies/mL pose an increased risk for fatal outcome.
  - Pediatric patients are more at risk for adenovirus
    - having higher viral load

BK polyoma virus

Polyomavirus infections

- BKV and JCV are commonly detected in the urine and blood of transplant patients, suggesting that immunosuppression allows latent infections to reactivate.
  - Infections are frequent in the second month after renal transplantation, but late infections, occurring several months or even years later, are not unusual.
  - BKV is most closely associated with kidney disease or cystitis in allograft recipients, either kidney transplant or BMT recipients

BK polyoma virus Diagnosis

BKV infection manifestation

- BK viruria, BK viremia and BKVN
  - BK viruria precedes BK viremia by a median of 4 weeks and BKVN by a median of 12weeks

Diagnostic techniques

- Urine cytology using Papanicolaou stains or on phase contrast microscopy
  - detection of 'decoy' cells in the urine
- BK viral load
  - In urine to monitor BKV infection
    - Low levels of viruria may reflect asymptomatic shedding
    - Increasing viral load is indicative of active BKV replication
    - Variables; fluctuations in urine content and the method of sample processing and shipment, can contribute to interassay variations
      - BK viral copy number depending on supernatants, cell pellets or resuspended urine are used for DNA preparation

.. BKV diagnosis

tests for BK viruria

- amplification of viral VP1 mRNA in urine may be a better test for BK viruria as it represents active BKV replication
  - Using a cutoff value of 6.5×10^5 BKV VP1 mRNA copies per nanogram total RNA
    - a 94% sensitivity and specificity for **BKVN**
  - a larger cohort study reported urinary BKV VP1 mRNA expression continued to accurately diagnose **BKVN**
    - **a non-invasive test for prediction of BKVN**

BK viremia; can be measured quantitatively by PCR in plasma

- **BKVN**
  - 5000 copies/mL; sensitivity of 100% / a false-positive diagnosis in 15.2%
  - >7700 copies/mL
  - 1000 copies/mL
- a threshold value that can accurately diagnose BKVN without the need for a biopsy does not exist

More examples of why you need clinical virologists to interpret results

- CMV in sputum: may not be at all important unless also found in blood
- adeno in blood: probably not requiring treatment unless increases by 1-2 logs in a day or until log 6
- BK in urine: unimportant in renal transplants because so common - becomes important once in blood
- HSV in blood: you would think it is important, but sometimes it is just a marker of high mortality risk

RNA Respiratory viruses

Diagnosis of Respiratory Viral Infection

- By combination of SEROLOGY, NUCLEIC ACID TESTING, and Histopathology.
- SEROLOGY
  - In general not useful for initial diagnosis.
  - Reduced sensitivity among transplant recipients.
- Virus isolation
  - Available for most of common RNA viruses except hMPV and Coronaviruses.
  - Special cell line and condition needed to grow the viruses.
  - Tend to be inefficient;
    - Depends on site of sampling
      - BAL and Nasal wash; greatest yield
  - Time consuming; dependent on virus, viral inoculums, cell line and growth condition from 3 –21 days.

Diagnosis of Respiratory Viral Infection

- Virus isolation cont.
  - Shell vial assay;
    - More rapid, earlier detection (24–48h)
    - Lower sensitivity compared to traditional culture method.
- Rapid antigen detection
  - Despite their speed (30–60 min);
    - Substantially lower sensitivity among immunocompromised patients specially adult.
      - RSV; 15% for nasal wash – 89% for BAL.
  - Direct fluorescence assay (DFA)
    - Limited by lack of reagents for some of viruses (hMPV, Rhino & Corona)
    - Appear to be less sensitive than PCR
- PCR-based Assay;
  - appear to be the most sensitive diagnostic tools available and most allow for simultaneous detection of a broad rang of respiratory pathogens from single sample.

Thanks for your patience