Monitoring of viral parameters in transplant patients

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Ghent University Hospital – Hasselt University

08/02/2011
Special considerations regarding infections in transplant recipients

- Susceptibility of the host/to the environment
  - High host susceptibility to infection + poor ability to combat it due to impaired cell-mediated immunity
  - High vulnerability to perturbations in the environment what produces exposure to pathogens not problematic to immunocompetent hosts
  - Need of recognition of geographic- or temporal clustering pathogen patterns
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- Broader spectrum of pathogens
  - Viruses (*Herpesviridae*), fungi (*Aspergillus, Pneumocystis*), intracellular bacteria (*Listeria, Mycobacterium*)
  - Specificity related to the type and/or transplanted organ
  - Characteristic time windows in which likelihood of infection with some pathogens becomes greater

*Linden PK, Infect Dis Clin North Am, 2009*
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  – Absence of expected local clinical and/or radiological signs of infection due to diminished or absent inflammatory responses
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Special considerations regarding infections in transplant recipients: *Herpesviridae*

- dsDNA; 120-230 kbp long
Special considerations regarding infections in transplant recipients: **Herpesviridae**

- dsDNA; 120-230 kbp long
- Enveloped viruses: envelope has various by virus encoded glycoproteins ⇒ role in viral binding to the receptors of the host
- Virions spherical to pleomorphic, 120-200 nm
Special considerations regarding infections in transplant recipients: *Herpesviridae*
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Specific feature of all herpesviruses is the capacity to establish latency in the host with periods of reactivation.
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- **Immunosuppression**
  - natural
  - jatrogenic
- **Internal stimuli**
  - fever
  - stress
  - menstruation
- **External stimuli**
  - UV light
  - trauma
Special considerations regarding infections in transplant recipients: **Herpesviridae**

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Special considerations regarding infections in transplant recipients: *Herpesviridae*

- Based on cellular types harboring latent virus
- Based on genomic sequence

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\alpha-, \beta-, \gamma- & \text{herpesvirus subfamilies}
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Special considerations regarding infections in transplant recipients: CMV

- **Epidemiology**
  - ~ 80% of world population is seropositive for CMV
    - low socioeconomic status > high socioeconomic status
  
  - Present in all body fluids and secretions (urine, saliva, blood, faeces, breastmilk, semen, cervical secretions)
  
  - Transmission by “long-term intimate exposure”:
    - Jong children in daycare centres
    - Trasfusion of blood and blood products
    - Vertical + breastfeeding
    - Sexuel contact
Special considerations regarding infections in transplant recipients: CMV

- Clinical picture

  CMV causes almost never a symptomatic infection in immunocompetent host

  - CMV disease ⇒
    - immunocompromised
      - solid organ transplants (SOT) (leber, haert, lung, kidney)
      - haematopoietic stem cell transplants (HSCT)
    - immunologically immature host
      - fetus
### Special considerations regarding infections in transplant recipients: CMV

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• Prevention of CMV disease in transplant recipients: why?
  – CMV disease = single most important infectious complication after solid organ transplantation (SOT)
  – CMV disease is a major cause of morbidity in SOT patients
  – CMV disease is a major complication of haematopoietic stem cell transplantation (HSCT)
  – Clinical manifestations: pneumonitis, hepatitis, retinitis, gastrointestinal or renal involvement, fever, myelosuppression,
  – CMV is associated with acute and chronic graft rejection
  – CMV is associated with bacterial and fungal superinfection
  – Interaction with other viruses
    • Accelerating of HCV pathogenesis
    • Increased incidence of EBV-related post-transplant lymphoproliferative disease

Razonable R. et al., Herpes, 2004
Special considerations regarding infections in transplant recipients: CMV

- Prevention of CMV disease in transplant recipients: how do patients get CMV infection?
  - Primary infection
    - When an allograft from a seropositive individual is transplanted into a seronegative recipient
    - > 90% become ill
  - Reactivation infection
    - Reactivation of endogenous latent infection
    - ~ 15% become ill
  - Superinfection
    - Both donor and recipient are seropositive
    - The reactivated virus is of donor origing
    - ~ 25% become ill
Special considerations regarding infections in transplant recipients: CMV

- Prevention of CMV disease in transplant recipients: when do patients get CMV infection?
  - If no therapeutic strategy involved CMV disease occurs 1-3 months
  - Emergence of late CMV disease
    - > 3 months posttransplant
    - Primary infection or reactivation
    - May be present with atypical symptoms
      - Diagnosis can be missed
      - Patient may not be followed by primary center or may not be followed as closely

Limaye AP et al., Lancet, 2000
Paya C et al., AJT, 2004
Special considerations regarding infections in transplant recipients: CMV

• Prevention of CMV disease in transplant recipients: are certain patients at risk?

Risk Factors for Cytomegalovirus (CMV) Disease in Transplant Recipients

- CMV serology
  - Donor CMV seropositivity in a seronegative solid organ transplant recipient (D+/R−)
  - Hematopoietic stem cell recipient CMV seropositivity (R+)
- Allogeneic stimulation
- Graft rejection (in SOT patients)
- Graft-versus-host disease (in HSCT patients)
- Certain types of solid organ transplantation
  - Kidney-pancreas
  - Lung
- Allogeneic transplantation (compared to autologous HSCT)
- Large viral load
- Concomitant viral infections
  - Human herpesvirus-6
  - Human herpesvirus-7
- Use of immunosuppressive drug therapy
  - Antilymphocyte globulin
  - Muromonab-CD3
  - Antithymocyte globulin
  - Corticosteroids
- Other immunosuppressive drugs (e.g., alemtuzumab, MMF)

CMV = cytomegalovirus, SOT = solid organ transplant, HSCT = hematopoietic stem cell transplant, MMF = mycophenolate mofetil, D+ = donor positive, R− = recipient negative, R+ = recipient positive.

Razonable R, Am J Health Syst Pharm, 2005
Special considerations regarding infections in transplant recipients: CMV

- Prevention of CMV disease in transplant recipients: viral dynamics
  - Viral load at the onset or peak of CMV reactivation correlates with the appearance of CMV disease in both HSCT and SOT patients
  - Risk of disease = viral load in whole blood > 5 log genomes/ml
  - “Threshold concept of CMV disease” = small increase in viral load corresponds with rapid increases in the probability of disease

Griffiths P et al., Herpes, 2008
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Preemptive approach = initiation of therapeutic action only after positivity of an indicator test before the onset of clinical symptoms
Special considerations regarding infections in transplant recipients

Preemptive approach = initiation of therapeutic action only after positivity of an indicator test before the onset of clinical symptoms
Special considerations regarding infections in transplant recipients: CMV

• Prevention of CMV disease in transplant recipients: use of indicator test (preemptive strategy!)
  – An accurate detection method to identify patients at risk for disease is an essential component of this strategy

• Widespread use of quantitative PCR methods
  – Next to use as indicator test in preemptive strategy can also:
    » Provide rapid and reliable diagnosis of established CMV disease
    » Monitor of response to antiviral therapy
    » Predict the risk of virological and clinical relapse
    » Serve as an early indicator or antiviral resistance
Special considerations regarding infections in transplant recipients: CMV

- Prevention of CMV disease in transplant recipients: use of indicator test
  - Quantitative CMV DNA measurements
  - Blood compartment: whole blood
  - Consider assay variability to determine true increases
    - The coefficient of variation of most PCR-based methods for viral loads close to the limit of detection may be as high as 30%
      - Increases less than 0.5 \( \log_{10} \) or 3 times the baseline level may not represent true increases
  - Frequency: ?
    - once weekly
    - high risk groups: twice weekly
  - Cut-off point for initiation of preemptive treatment: ?
    - Vary accordingly to the patient group
      - Related versus unrelated donors
      - Type of transplant
    - Haematological procedure used
      - Bone marrow versus peripheral blood stem cells

Boeckh M & Ljungman P, Blood, 2009
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- Prevention of CMV disease in transplant recipients: use of indicator test: cut-off point for initiation of preemptive treatment
  - **Definition of clinically validated thresholds for initiating preemptive treatment in SOT and HSCT recipients is a major goal in transplantation setting**
  - Retrospective analysis of viral load values at the time of initiation of preemptive therapy based on defined antigenemia cut-offs
    - >90% of SOT treated patients: CMV DNA levels in whole blood > 300,000 copies/ml
    - Positive predictive value 90.9%
    - Negative predictive value 95.8%
    - >90% of untreated SOT patients: CMV DNA levels < 300,000 copies/ml
    - 19.6% of HSCT treated patients: CMV DNA levels in whole blood > 10,000 copies/ml
    - Positive predictive value 91.7%
    - Negative predictive value 28.6%
    - 94.7% of untreated HSCT patients: CMV DNA levels < 10,000 copies/ml

*Baldanti F et al., JCV, 2008*
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**DNAemia cut-off = same clinical results as antigenemia cut-off**

**PPV: patients treated under antigenemia cut-off would be also treated under DNAemia cut-off**

**NPV: 1/3 patients treated under antigenemia have the same levels of CMV DNA as untreated patients**
Special considerations regarding infections in transplant recipients: CMV

• Prevention of CMV disease in transplant recipients: use of indicator test
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Special considerations regarding infections in transplant recipients: CMV

- Prevention of CMV disease in transplant recipients: use of indicator test: standardization of cut-off values
Special considerations regarding infections in transplant recipients: CMV

- Prevention of CMV disease in transplant recipients: use of indicator test: **standardization of cut-off values**
  - Multicenter quality control study
  - Fifteen Italian viral diagnostic laboratories from different transplantation centers
  - Participation in EQA Programme for CMV DNA from QCMD
  - Two in-house and 5 commercial methods for CMV DNA quantification
  - Specificity: 100%
  - Sensitivity: 100% for samples > 1 000 copies/ml
  - Variability range: 0.5 log\(_{10}\) for samples >= 5 000 copies/ml
  - **An acceptable level of variability was reached among different in-house and commercial methods for CMV DNA quantification in samples containing a clinically significant viral DNA amount ⇒ standardized cut-offs established for preemptive therapy in different transplantation centres should provide comparable clinical and virological results among centers**

*Lilleri D et al., New Microbiol, 2009*
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MORPHOLOGICAL AND BIOLOGICAL STUDIES ON A VIRUS IN CULTURED LYMPHOBLASTS FROM BURKITT’S LYMPHOMA

By M.A. EPSTEIN. M.D. G. HENLE, M.D., B.G. ACHONG, M.B.,B.CH., AND Y.M. BARR
The Journal of Experimental Medicine
Vol. 121 No 4 April 1, 1965
Special considerations regarding infections in transplant recipients: EBV

- **Primary infection:**
  - Respiratory transmission (intiem contact (kissing), saliva)

- Infection of mouth epithelium $\Rightarrow$ productive infection $\Rightarrow$ infection of B-lymphocytes $\Rightarrow$ latent

- **Symptomatology:**
  - Children: mostly subclinical
  - Adolescents: mononucleosis infectiosa (general malaise, pharyngitis, atypical lymphocytosis, generalized lymphoadenopathy, splenomegaly)
Special considerations regarding infections in transplant recipients: EBV

• **Latency:**
  
  – EBV-infected B lymphocytes are immortalized ⇔ non-EBV-infected B-lymphocytes die in 1 week in viral culture)
  
  – Immortalization is an important step in cellular transformation ⇒ tumoral predisposition
  
  – Clinical pictures: Burkitt lymphoma, nasopharyngeal carcinoma, posttransplantation lymphoproliferative disorders (PTLD)
Special considerations regarding infections in transplant recipients: EBV

- **Facts about PTLD:**
  - The *incidence* of PTLD ranges from 2 to 20% depending in part on the type of transplanted organ, immunosuppressive regimen and EBV serology before transplant
  - PTLD can *present* as:
    - Localized disease, sometimes involving the transplant organ
    - Mononucleosis-like syndrome
    - Disseminated lymphoma
  - The *histological pattern* is widely heterogeneous, ranging from polymorphic lympho-proliferation to monomorphic lymphoma
  - Reduction or withdrawal of immunosuppression induces *regression* of PTLD in 20-80%
    - Graft rejection occurs in >40% of patients as a consequence of this approach and represents the major limitation of this intervention

*Green M., Am J Transplant, 2001*
*Straathof K. et al., Br J Haematol, 2002*
Special considerations regarding infections in transplant recipients: EBV

- **EBV pathogenesis: why transplant recipients are at risk for PTLD?**
  - In healthy subjects:
    - Cellular immunity appears rapidly during EBV primary infection and is probably the most important defense against this virus, keeping it silent despite long-life persistence in B lymphocytes *(Smets F. et al., Transplantation, 2002)*
    - The main part of this immunity is based on CD8 cytotoxic T cells targeted against EBV antigens *(Smets F. et al., J Hepatol, 2000)*
Special considerations regarding infections in transplant recipients: EBV

- **EBV pathogenesis:** why transplant recipients are at risk for PTLD?
  - In transplant patients:
    - Risk of developing PTLD is associated to primary EBV infection and intense immunosuppression (Sokal E. et al., Transplantation, 1997)
    - No PTLD observed (also in children) in patients EBV-seropositive before transplant (Yang J. et al., Blood, 2000)
    - If PTLD observed (Yang J. et al., Blood, 2000)
      - low incidence
      - later post-transplant
      - different EBV-related pathogenesis?
Special considerations regarding infections in transplant recipients: EBV

- Prevention of PTLD in transplant recipients: why pediatric patients are specifically at risk for PTLD?
  - Epidemiology of primary EBV:
    - Low socioeconomic status: infection is almost universally acquired in early childhood and is usually subclinical
    - High socioeconomic status: subjects are often infected in adolescence and early adult life when 23-47% of infectious result in infectious mononucleosis
  - Due to their young age, most pediatric transplant candidates do not have EBV immunity at the time of transplant
  - Most of them undergo primary EBV infection in the first 3 months after transplantation (Smets F. et al., J Hepatol, 2000)
  - PTLD is the commonest form of post-transplant malignancy in paediatric transplant recipients (Penn I., Transplant Sci, 1994)
Special considerations regarding infections in transplant recipients: EBV

- Prevention of PTLD in transplant recipients: when does PTLD occur in transplant patients?
  - Bimodal distribution:
    - Early peak occurring within 2 years of transplantation
      - Associated with primary EBV infection
      - Generally responds to a reduction in immunosuppression
      - Median time of onset: 6-10 months
    - Later peak occurring > 2 years post-transplantation
      - Independent of EBV status or immunosuppression
      - Usually not responsive to a reduction in immunosuppression
      - Median time of onset: 50-60 months

-Lim W. et al., Nephrology, 2006
-Savoldo B. et al., Am J Transplantation, 2005
Special considerations regarding infections in transplant recipients: EBV

- Treatment of PTLD:
  - Anti-B-lymphocyte antibodies
    - Humanized anti-CD20 monoclonal antibody (Rituximab)
    - First introduced for the treatment of non-Hodgkin’s follicular lymphomas
  - Safety and efficacy of Rituximab for treatment of PTLD affecting both HSCT and SOT recipients
  - Mechanism of action: elimination of normal B cells as well as EBV-infected and/or transformed lymphocytes, predominately by in vivo complement activation and antibody-dependent cellular toxicity
  - Overall, Rituximab allows the achievement of complete regression of PTLD in 50-60% of patients

*Cook R. et al., Lancet, 1999*
*Verschuuren E. et al, Transplantation, 2002*
Special considerations regarding infections in transplant recipients: EBV

- **Diagnosis of PTLD: why not serology?**
  - Aspecific antibodies: heterophile antibodies (HA)
    - HA are commonly used in the diagnosis of infectious mononucleosis (IM)
    - HA are antibodies that are generated to an antigen of one species but are crossreactive with an antigen in another species
    - Some of the antibodies generated during the course of IM can bind to the red blood cells of other animals, especially horse and sheep
    - Diagnosis of IM is based on the detection of HA that would agglutinate the horse, sheep or bovine erythrocytes
    - Lack sensitivity, especially in children (Sumaya C. and Ench Y., Pediatrics, 1985)
    - Persistent at low levels for up to 1 year (Blake J. et al., J Clin Pathol, 1976)
Special considerations regarding infections in transplant recipients: EBV

• Diagnosis of PTLD: why not serology?
  – EBV specific antibodies
Special considerations regarding infections in transplant recipients: EBV

• Diagnosis of PTLD: why not serology?
  – False positive and false negative IgM and false negative IgG results have been well documented \((\text{Robertson P. et al., J Med Virol, 2003})\)
  – Presence of serum IgG anti EA-Ab is not a reliable marker of active EBV infection in renal transplant patients \((\text{Merlino C. et al., New Microbiol, 2001})\)
  • Serological reactivity without viral replication
    – 40% of EA-Ab positive patients \(\Rightarrow\) undetectable EBV DNA
  • Viral replication without serological reactivity
    – 71% of EA-Ab negative patients \(\Rightarrow\) detectable EBV DNA
Special considerations regarding infections in transplant recipients: EBV

- Prevention of PTLD in transplant recipients: what is the specimen of choice to determine EBV viral load?
  - EBV-driven lymphoproliferation is sustained by expression in infected cells of latency-associated antigens and is not associated with the lytic (active) phase of infection
  - The EBV lytic cycle is induced following differentiation of latently infected memory B-cells in plasma cells, and virus progeny is released into body fluids

**Determination of cell-associated EBV DNA loads = marker of EBV-induced cell proliferation**

**Determination of cell-free EBV DNA loads = marker of either viral production or release of episomal DNA from apoptotic cells, or both**

–Laichalk L and Thorley-Lawson D., J Virol, 2005
Special considerations regarding infections in transplant recipients: EBV

- Prevention of CMV disease in transplant recipients: what is the specimen of choice to determine EBV viral load?
  - Cell-associated EBV DNA:
    - B-cells
    - Peripheral blood mononuclear cells (PBMC)
  - Cell-free EBV DNA:
    - Plasma
  - Cell-free + cell-associated EBV DNA:
    - Whole blood
Special considerations regarding infections in transplant recipients: EBV

- Prevention of CMV disease in transplant recipients: what is the specimen of choice to determine EBV viral load?
  - **Cell-associated EBV DNA:**
    - B-cells: most sensitive ⇔ too labour intensive (Wagner H et al., Klin Padiatr, 2000)
    - Peripheral blood mononuclear cells (PBMC): high sensitivity ⇔ very labour intensive
  - **Cell-free EBV DNA:**
    - Plasma
  - **Cell-free + cell-associated EBV DNA:**
    - Whole blood
Special considerations regarding infections in transplant recipients: EBV

- Prevention of PTLD in transplant recipients: what is the specimen of choice to determine EBV viral load?
  - Technological progress:
  - Real-time PCR techniques
  - Automated extraction procedures
  - Availability of international quality control panels

More reproducible quantification of EBV DNA

Quantification of EBV DNA in plasma or whole blood ⇒ technical simplification of diagnostic procedures since PBMC separation before DNA extraction would be avoided
Special considerations regarding infections in transplant recipients: EBV

- Prevention of CMV disease in transplant recipients: what is the specimen of choice to determine EBV viral load? Whole blood?
  - Similar kinetics of EBV DNAemia in PBMC and whole blood (Hakim H. et al., J Clin Microbiol, 2007; Baldanti F. et al., J Clin Microbiol, 2008)

Monitoring of EBV DNA levels in whole blood is a valuable alternative to determination of EBV DNA levels in PBMC in the follow-up of transplant recipients
Special considerations regarding infections in transplant recipients: EBV

- Prevention of PTLD in transplant recipients: what is the specimen of choice to determine EBV viral load? Plasma?
  - Sensitivity of EBV DNA in PBMC and plasma are equal, specificity of EBV DNA is higher in plasma than in PBMC (100% ↔ 89%) (Wagner H. et al., Transplantation, 2001)
    - Cell-free EBV DNA better reflects response to therapy?

Monitoring of EBV DNA levels in plasma is successfully used in the follow-up of transplant recipients: importance of single centre experience
Special considerations regarding infections in transplant recipients: EBV

• Prevention of PTLD in transplant recipients: what are EBV viral load values in PTLD-patients?
  – pediatric liver transplant patients: all > 25,000 copies/µg DNA in PBMC (Smets F. et al., Transplantation, 2002)
  – pediatric liver transplant patients: all > 4,000 copies/µg DNA in PBMC (Savoldo B. et al., Am J Transplantation, 2005)
  – SOT: all > 500 copies/10^5 lymphocytes (Riddler S. et al., Blood, 1994)

  High

• Prevention of PTLD in transplant recipients: reporting units?
  – Correlation between copies/µg DNA and copies/ml in whole blood (Pang X. et al., 12th Annual ESCV Meeting, 2009)
Special considerations regarding infections in transplant recipients: EBV

• Prevention of PTLD in transplant recipients: what is the specimen of choice to determine EBV viral load?

Many groups are actively working on the identification of virologic markers predicting the development of PTLD with the objective of optimizing treatment options

Biological and technical issues contribute to the difficulty of reaching a consensus

No consensus on optimal specimen/cut-off value
No standardisation of PCR assays between centres
Special considerations regarding infections in transplant recipients: EBV

- Prevention of PTLD in transplant recipients: interlaboratory comparison of EBV viral load assays
  - Multicenter quality control study
  - Thirty viral diagnostic laboratories from different transplantation centers in USA (17), Canada (11) and Europe (2)
  - Variation observed on individual samples ranged from 2.28 $\log_{10}$ to 4.14 $\log_{10}$
  - Variation was independent of dynamic range and use of commercial versus laboratory-developed assays
  - Only 47% of results within acceptable level of variability of 0.5 $\log_{10}$
  - Interlaboratory variability on replicate samples was significantly higher than intralaboratory variability ($p < 0.0001$)

Kinetics of change in viral load appears more relevant than absolute values

Preiksaitis J. et al., Am J Transplant, 2009
Thank you for your attention!